Mechanosensitive mechanisms in transcriptional regulation

Akiko Mammoto[1,*], Tadanori Mammoto[1,*] and Donald E. Ingber[1,2,3,‡]

[1]Vascular Biology Program, Children's Hospital and Harvard Medical School, Boston, MA 02115, USA
[2]Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA 02115, USA
[3]Harvard School of Engineering and Applied Sciences, Cambridge, MA 02139, USA

*These authors contributed equally to this work
‡Author for correspondence (don.ingber@wyss.harvard.edu)

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Summary

Transcriptional regulation contributes to the maintenance of pluripotency, self-renewal and differentiation in embryonic cells and in stem cells. Therefore, control of gene expression at the level of transcription is crucial for embryonic development, as well as for organogenesis, functional adaptation, and regeneration in adult tissues and organs. In the past, most work has focused on how transcriptional regulation results from the complex interplay between chemical cues, adhesion signals, transcription factors and their co-regulators during development. However, chemical signaling alone is not sufficient to explain how three-dimensional (3D) tissues and organs are constructed and maintained through the spatiotemporal control of transcriptional activities. Accumulated evidence indicates that mechanical cues, which include physical forces (e.g. tension, compression or shear stress), alterations in extracellular matrix (ECM) mechanics and changes in cell shape, are transmitted to the nucleus directly or indirectly to orchestrate transcriptional activities that are crucial for embryogenesis and organogenesis. In this Commentary, we review how the mechanical control of gene transcription contributes to the maintenance of pluripotency, determination of cell fate, pattern formation and organogenesis, as well as how it is involved in the control of cell and tissue function throughout embryogenesis and adult life. A deeper understanding of these mechanosensitive transcriptional control mechanisms should lead to new approaches to tissue engineering and regenerative medicine.

This article is part of a Minifocus on Mechanotransduction. For further reading, please see related articles: 'Deconstructing the third dimension – how 3D culture microenvironments alter cellular cues' by Brendon M. Baker and Christopher S. Chen (*J. Cell Sci.* **125**, 3015-3024). 'Finding the weakest link – exploring integrin-mediated mechanical molecular pathways' by Pere Roca-Cusachs et al. (*J. Cell Sci.* **125**, 3025-3038). 'Signalling through mechanical inputs – a coordinated process' by Huimin Zhang and Michel Labouesse (*J. Cell Sci.* **125**, 3039-3049). 'United we stand – integrating the actin cytoskeleton and cell–matrix adhesions in cellular mechanotransduction' by Ulrich S. Schwarz and Margaret L. Gardel (*J. Cell Sci.* **125**, 3051-3060). 'Molecular force transduction by ion channels – diversity and unifying principles' by Sergei Sukharev and Frederick Sachs (*J. Cell Sci.* **125**, 3075-3083).

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Introduction

Tissue and organ architecture are constructed as a result of the complex orchestration of various cell types that exhibit specific behaviors (e.g. growth, differentiation, migration and apoptosis) in specific locations at precise times during embryogenesis. Transcriptional programs confer and maintain cellular identity and functions that are necessary for the maturation of embryonic cells into terminally differentiated tissues or organs, as well as for stem cell fate switching throughout adult life. These behaviors are regulated by turning genes on and off in a highly spatiotemporally controlled manner, and this process is mediated at the level of gene transcription through the interplay between RNA polymerase II, various transcription factors and their co-regulators (Box 1). Whereas most studies on transcriptional regulation have focused on genetic or chemical control mechanisms of transcription, recent work suggests that mechanical forces (Box 2) are equally important regulators of transcriptional control in embryonic and adult tissues. In this Commentary, we review these new insights into cellular mechanotransduction, and discuss how mechanical cues influence transcriptional regulation to govern organogenesis and to ensure tissue maintenance throughout adult life.

Mechanical determinants of transcriptional control during early embryogenesis

During development, individual cells sense changes in mechanical forces and transduce them into intracellular signals that drive alterations in cell growth, differentiation, migration and apoptosis that are required for tissue and organ development (reviewed in Farge, 2011; Mammoto and Ingber, 2010; Wozniak and Chen, 2009). Early in mammalian embryogenesis, the totipotent inner cell mass (ICM) of the blastocyst gives rise to embryonic stem cells (ESCs) as a result of the expansion of the blastocoel and secretion of liquid into the cavity. The accumulation of liquid pushes the ICM against the surrounding zona pellucida, and the ICM establishes a stable transcriptional regulatory circuit, whereby specific sets of transcription factors, including OCT4 (also known as POU5F1), SOX2 and NANOG, promote pluripotency (Rossant and Tam, 2009) (Fig. 1). Importantly, dense cultures of compact, rounded mouse and human ESCs exhibit higher expression of these pluripotent transcription factors than flattened (sparse) ESCs cultured in vitro (Hemsley et al., 2011; Peerani et al., 2007). Moreover, cell stretching induced by cyclic mechanical strain leads to downregulation of OCT4 gene expression in mouse ESCs, and

this is dependent on the level of cytoskeletal tension inside the cell (Chowdhury et al., 2010). These findings suggest that the transcriptional regulation that is necessary for pluripotency can be maintained and modified by the local micromechanical environment in the embryo.

Mechanical cues determine tissue pattern and size in the embryo

Studies of later developmental stages in the *Drosophila* wing have revealed that morphogen gradients determine tissue size and pattern, and that this process is mediated at the transcriptional level (Schwank and Basler, 2010). However, internal genetic programs and chemical cues do not appear to be sufficient to explain how these complex tissues form. Importantly, changes in the physical shape of the embryo can feed back to individual cells to mechanically control patterns of gene expression, and thereby govern tissue patterning during *Drosophila* morphogenesis (Farge, 2003). For instance, physiological deformations in embryonic *Drosophila* tissues caused by germband extension (GBE) triggers Src42A-dependent nuclear translocation of β-catenin from cell–cell junctions in the anterior pole of stomodeal cells, and activates the transcription of the basic helix-loop-helix transcription factor *Twist*, which is involved in pattern formation and is crucial for subsequent midgut differentiation (Desprat et al., 2008) (Fig. 2A).

Gastrulation in *Drosophila* is controlled by Twist in concert with the zinc-finger transcription factor Snail through Twist-dependent transcription of the gene encoding *Folded gastrulation* (*Fog*), and this process begins with mesoderm invagination (Seher et al., 2007) (Pouille et al., 2009) (Fig. 2B). First, Snail induces stochastic apical constrictions that are pulsed, and are subsequently stabilized by the secreted Fog protein (Martin et al., 2009). Mechanical tension that is generated in the apical membrane of the mesodermal cells by Snail-dependent constrictions inhibits Fog endocytosis, thereby enhancing the extracellular concentration and activating signaling pathways downstream of Fog (Pouille et al., 2009). These signals lead to local activation of the Rho and Rho-associated, coiled-coil containing protein kinase 1 (Rho–ROCK) pathway and myosin II (Myo-II) at the apical membrane, which subsequently drives mesoderm invagination (Dawes-Hoang et al., 2005). Twist is known to also activate another downstream signaling molecule, transmembrane protein T48, which reinforces apical constriction and subsequent invagination (Kolsch et al., 2007). Importantly, Myo-II, which is recruited locally in response to mechanical distortion, disappears from the membrane when mechanical tension is relieved during axis elongation in *Drosophila* (Fernandez-Gonzalez et al., 2009). These observations suggest that mechanical forces act in concert with chemical cues to orchestrate transcriptional and biochemical controls that are crucial for the formation of spatiotemporal patterns during early embryogenesis.

Mechanical cues also contribute to the control of cell proliferation, tissue growth and organ size during *Drosophila* development. For instance, computational modeling studies suggest that cells in the central parenchyma of the growing *Drosophila* wing become compressed, a cue that mechanically feeds back to suppress cell growth in the central area (Chen et al., 1997). Meanwhile, cell growth at the periphery of the wing will slow when the cells extend beyond the edges of the morphogen gradient. As a result, the model predicts that the tissue will grow uniformly (Hufnagel et al., 2007; Shraiman, 2005).

The Hippo pathway, a conserved signalling pathway that is crucial for the correct regulation of organ growth in *Drosophila* and vertebrates, appears to be involved in this feedback loop. The pathway is composed of the Hippo (Hpo) and Warts (Wts) kinases, together with their co-factors scaffold protein salvador (Sav) and MOB kinase activator-like 1 (Mats), and the transcriptional co-activator Yorkie (Yki; Yap1 in mammals) (Dick and Mymryk, 2011; Zhao et al., 2010). In both *Drosophila* and mice, active Hippo signaling results in phosphorylated Yki (and Yap1, respectively) being retained in the cytoplasm, which leads to transcriptional downregulation of target genes, such as the cell cycle regulator cyclin E (Fig. 2C) (Dong et al., 2007; Oh and Irvine, 2008; Oh and Irvine, 2009; Ren et al., 2010). This pathway, which enables cells to alter their proliferative rate in response to external mechanical cues, is sensitive to mechanical inputs from the extracellular matrix (ECM) and cell–cell contacts, and it requires an intact actin cytoskeleton (Assoian and Klein, 2008; Klein et al., 2007). Consistent with this observation, changes in the

Box 1. Regulation of transcription

Transcription: the synthesis of an RNA molecule from a complementary DNA sequence by RNA polymerase II that binds to the promoter region of a gene.
Transcription factor: regulatory protein that regulates transcription by binding with high affinity to its target promoter sequence on the DNA. Alone or in combination with other associated proteins, transcription factors promote or block recruitment of RNA polymerase II to the promoter regions of a gene.
Transcriptional regulation: controls the rate of transcription by regulating the binding of RNA polymerase II to the DNA strand.
Co-activator or co-repressor: associate with transcription factors to, respectively, up- or downregulate the rate of transcription.

Box 2. Mechanical forces involved in regulation of transcription

Hydrostatic pressure: mechanical force applied by fluids or gases (e.g. blood or air) that perfuse or infuse living organs (e.g. blood vessels or lung).
Shear stress: frictional force of fluid flow on the surface of cells. The shear stress generated by the heart pumping blood through the systemic circulation has a key role in the determination of the cell fate of cardiomyocytes, endothelial cells and hematopoietic cells.
Compressive force: pushing force that shortens the material in the direction of the applied force.
Tensional force: pulling force that lengthens materials in the direction of the applied force.
Cell traction force: is exerted on the adhesion to the ECM and other cells as a result of the shortening of the contractile cytoskeletal actomyosin filaments, which transmit tensional forces across cell surface adhesion receptors (e.g. integrins, cadherins).
Cell prestress: stabilizing isometric tension in the cell that is generated by the establishment of a mechanical force balance within the cytoskeleton through a tensegrity mechanism. Pulling forces generated within contractile microfilaments are resisted by external tethers of the cell (e.g. to the ECM or neighboring cells) and by internal load-bearing structures that resist compression (e.g. microtubules, filipodia). Prestress controls signal transduction and regulates cell fate.

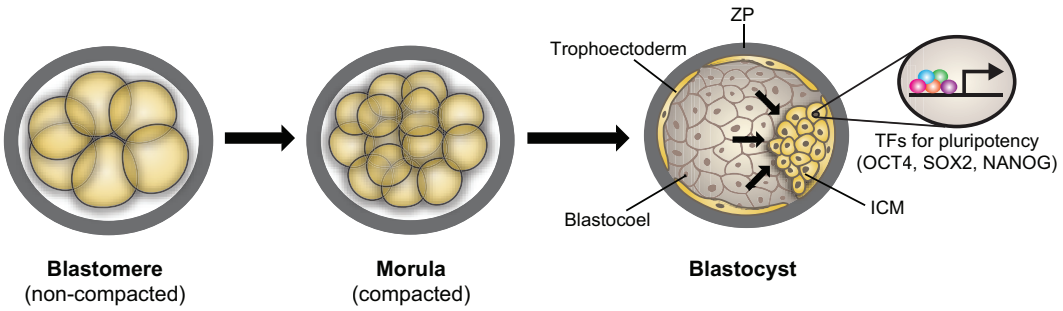

Fig. 1. Mechanical transcriptional control and pluripotency. The expanding blastocoel pushes the inner cell mass (ICM) against the outer zona pellucida (ZP), which induces specific transcription factors (TFs) that are crucial for pluripotency.

physical organization of the actin cytoskeleton can also alter cell growth by modulating Hippo signaling through Yap1. This further highlights a role for this cytoskeleton-dependent mechanical signaling pathway in transcription regulation, which leads to the inhibition of cell growth in response to environmental stimuli (Sansores-Garcia et al., 2011).

Mechanical control of organ-specific determination of the cell fate during development

Morphogen gradients in the embryo are converted into patterns of gene expression by concentration-specific responses of target genes (Wolpert, 1969). These gradients spatially limit the expression of target-differentiation genes and produce distinct organ-specific cell fates within precise physical boundaries of tissues (Small et al., 1992). Recent evidence suggests that external mechanical cues also contribute to the determination of tissue boundaries and organ-specific cell fates. This is important because maintaining the boundaries that are defined by these expression patterns is crucial for the maturation of these early patterns into stable tissue structures during embryogenesis (Dahmann et al., 2011). Studies in *Drosophila* have revealed that cytoskeletal tension – which is controlled by Rho, ROCK and Myo-II, and exerts tension on cell–cell adhesions – contributes to the formation of these discrete compartmental boundaries (Landsberg et al., 2009; Monier et al., 2010). Furthermore, during tooth formation in mouse, the dental epithelium produces morphogen gradients of opposing attractive and repulsive motility factors (FGF8 and semaphorin 3F, respectively) that generate defined areas of cell compaction in a process known as the 'formation of the condensed mesenchyme'. This, in turn, triggers the protein expression of sets of transcription factors that orchestrate organ-specific morphogenesis as a result of cell distortion and subsequent inhibition of Rho signaling (Mammoto et al., 2011) (Fig. 2D). Thus, developmental patterning and organ-specific determination of the cell fate seem to be governed through a complex mechanochemical mechanism in which chemical cues manifest their actions largely through changes in the physical microenvironment that feed back into chemical signals – which subsequently alter transcriptional control. This theory is supported by observations during mouse mammary gland development: reorganization of mouse mammary epithelial cells into three-dimensional (3D) polarized acinar structures, which are induced by the ECM protein laminin, exposes prolactin receptors to the local prolactin hormone. This, and following the nuclear translocation of the transcription factor STAT5, activates the receptors and leads to β-casein expression, which induces differentiation of cells into the mammary-specific cell lineage (Xu et al., 2009).

Mechanosensitive transcriptional control during organ development and homeostasis

Mechanical forces not only have a role during developmental processes, but are also crucial for maintaining the function of most adult organs and organ systems, including heart (Groenendijk et al., 2007; McCain and Parker, 2011), blood vessels (Culver and Dickinson, 2010; Shiu et al., 2005), lungs (Hooper and Wallace, 2006), hematopoietic system (Adamo et al., 2009; North et al., 2009), gastrointestinal tract (Gayer and Basson, 2009) and musculoskeletal system (Huang and Ogawa, 2010; Tidball, 2005). Below, we provide examples of this form of mechanoregulation in relevant organ systems.

Heart

GATA-binding protein 4 (GATA4) is a transcription factor that regulates heart development (Bruneau, 2002), and is activated by vasopressin-induced increases in cardiac afterload and through direct stretching of the ventricles in rats (Fig. 3) (Hautala et al., 2001; Hautala et al., 2002). GATA4 has also been implicated as a mechanical load-responsive mediator of transcription that regulates the expression of genes that contribute to cardiac remodeling and ventricular hypertrophy, such as B-type natriuretic peptide (BNP) (Marttila et al., 2001).

Formation of the heart valve is also flow-dependent (Bartman et al., 2004; Hove et al., 2003; Wirrig and Yutzey, 2011) and GATA4, which is also activated by the changes in blood flow in the heart (Huang et al., 2010), appears to partly mediate this response. For example, GATA4 interacts with SMAD4, another transcription factor that is part of the bone morphogenetic protein (BMP) and transforming growth factor β (TGF-β) TGF-β signaling cascade. In mouse, they co-regulate valve formation by controlling endocardial cushion development (Moskowitz et al., 2011). In zebrafish, Krüppel-like factor 2 (KLF2) – another flow-sensitive transcription factor that orchestrates the expression of angiogenic, antithrombotic and atheroprotective genes in endothelial cells (Lee et al., 2006) – has been shown to have crucial roles in valve morphogenesis (Vermot et al., 2009).

Hematopoietic system

Interestingly, in the mouse embryo, the initiation of heart pumping and blood flow also switches on the formation of blood cells, activating hematopoietic stem cells in the

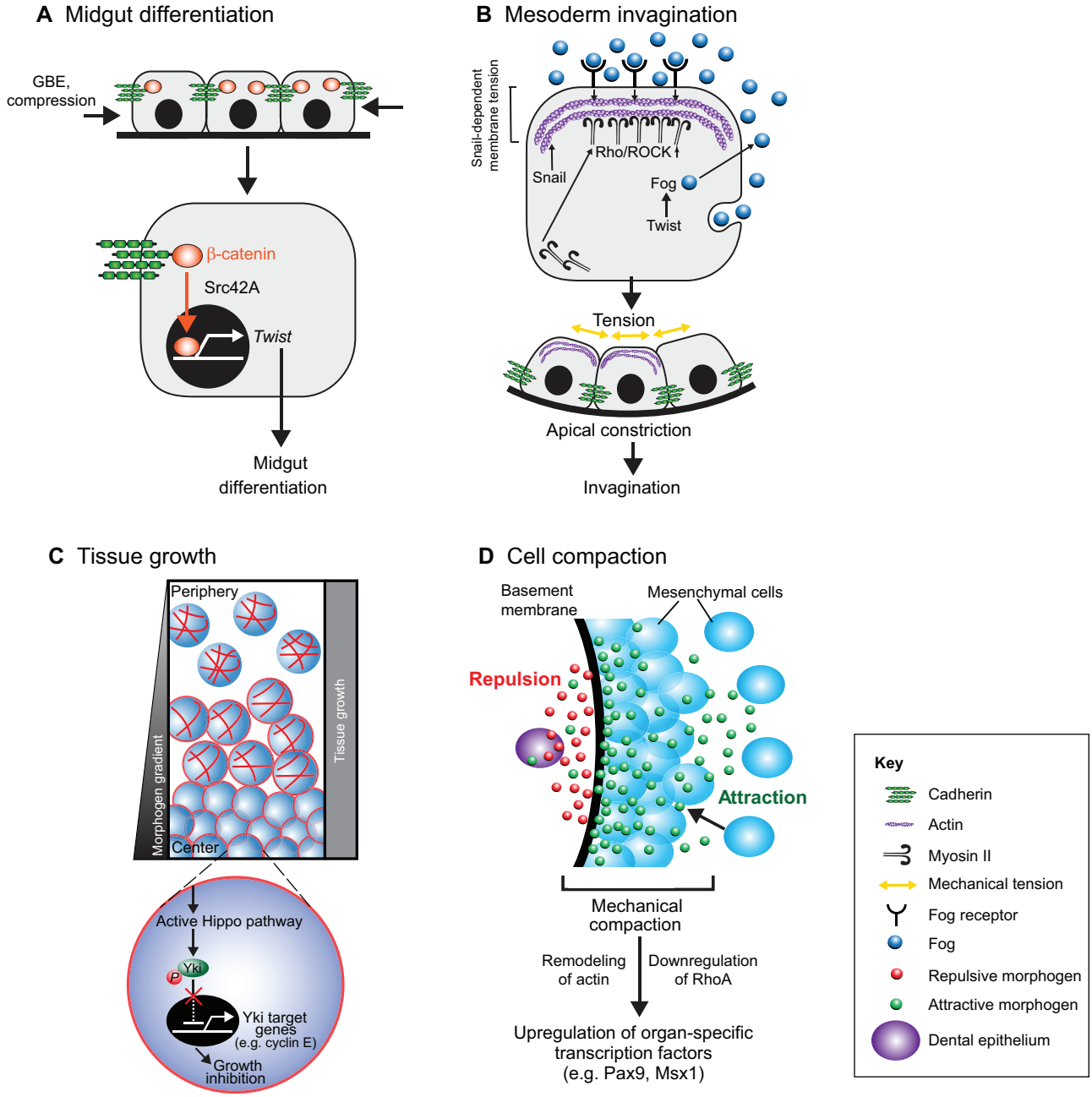

Fig. 2. Mechanical control of transcription during embryonic pattern formation. (**A**) Midgut differentiation. In *Drosophila* anterior stomodeal cells, mechanical compression as a result of germ band extension (GBE) induces expression of the gene encoding the transcription factor Twist, which is crucial for midgut differentiation through a Src42A-dependent mechanotransduction process that leads to the translocation of β-catenin into the nucleus. Modified with permission from Mammoto and Ingber, 2010 (Mammoto and Ingber, 2010). (**B**) Mesoderm invagination. In the *Drosophila* mesoderm, the Snail-dependent unstable pulses of apical constriction lead to a mechanically induced inhibition of Fog endocytosis through an increase in membrane tension. This activates the Fog–Rho–ROCK–Myo II signaling pathway, which leads to stable apical constriction and invagination. Fog is expressed under the control of Twist. Modified with permission from Mammoto and Ingber, 2010 (Mammoto and Ingber, 2010). (**C**) Tissue growth. During wing growth in *Drosophila*, cells in the central regions are compressed. This results in remodeling of actin in these cells, which activates the Hippo pathway and leads to phosphorylation of the transcription factor Yki, which prevents its translocation into the nucleus. Consequently, transcriptional activity of Yki target genes, such as cyclin E, is decreased and cell growth is inhibited. At the same time, cells at the periphery slow their proliferation because they have grown beyond the edges of the morphogen gradient. By employing these two mechanisms, the whole tissue grows uniformly. (**D**) Cell compaction. During teeth development in mouse, the early dental epithelium (DE) produces the antagonistic morphogens FGF8 (green) and semaphorin 3f (red) that attract and repulse mesenchymal cells, respectively. This causes mesenchymal cells to pack tightly during mesenchymal condensation in tooth development. The resultant mechanical compaction-induced changes in cell shape induce sets of transcription factors that are crucial for organ-specific morphogenesis.

aorta–gonad–mesenephron (AGM) region, the origin of the dorsal aorta (Medvinsky and Dzierzak, 1996). This effect is mediated by the transcription factor RUNX1 that is induced by flow-sensitive production of nitric oxide (NO) in mice and zebrafish (Fig. 3) (Adamo et al., 2009; Lichtinger et al., 2010; North et al., 2009). Thus, mechanical forces generated by blood flow are central to the control of transcriptional activities that govern development of the circulatory system.

Blood vessels

Endothelial cells within blood vessels are constantly exposed to fluid shear stresses, pulsatile pressures and cyclic mechanical strain. The effects of fluid shear on the transcription of genes that encode proteins that are crucial for vascular homeostasis, such as platelet-derived growth factor B (PDGFB) (Resnick et al., 1993), nitric oxide synthase 3 (eNos, also known as NOS3) (Davis et al., 2004) and platelet endothelial cell adhesion molecule 1 (PECAM1) (Almendro et al., 1996), are regulated by shear-stress-responsive elements (SSREs) within their promoter regions. Fluid shear stress regulates binding of mechanosensitive transcription factors, such as nuclear factor kappa B (NF-κB) and early growth response 1 (EGR1), to SSREs in endothelial cells, where they maintain blood vessel homeostasis (Gimbrone et al., 2000). Endothelial cells also sense shear stress through a mechanosensory cell–cell adhesion complex containing PECAM-1, vascular endothelial (VE)-cadherin and vascular endothelial growth factor 2 (VEGFR2); flow-mediated mechanical distortion of the endothelial cells activates NF-κB through the integrin–p38 MAPK (also known as MAPK14) signaling cascade (Orr et al., 2005; Tzima et al., 2005). Activated NF-κB upregulates the expression of genes that encode proteins such as eNOS (Balligand et al., 2009; Davis et al., 2004; Ozawa et al., 2004), several cytokines (Allport et al., 2000; Martin et al., 1997) and adhesion molecules that are crucial for maintaining vascular homeostasis (Ahmad et al., 1998). In addition, fluid shear stress activates the transcription of the gene encoding VEGFR2 through binding of the transcription factor SP1 to its promoter, and this transcriptional regulation is crucial for endothelial cell survival (Urbich et al., 2003).

During angiogenesis, VEGFR2 protein synthesis and the formation of new microvessels are regulated by changes in ECM mechanics (e.g. stiffness or compliance), which alter the balance of activities between two transcription factors GATA2 and TFII-I (also known as GTF2I) in endothelial cells (Mammoto et al., 2009). In vascular smooth muscle cells, remodeling of the arteries in response to mechanical loads – such as changes in blood pressure – is also regulated at transcriptional level by the mechanosensitive transcription factor EGR1 (Morawietz et al., 1999). Mechanical strain induces transcription of the cysteine-rich angiogenic inducer 61 (*CYR61*), a crucial gene for vascular development and repair in smooth muscle cells (Chaqour and Goppelt-Struebe, 2006). In addition, cyclic stretching of rat aortic smooth muscle cells has been shown to increase the protein expression of endothelin B receptor, which is involved in blood-pressure-dependent arterial remodeling. At the transcriptional level this is mediated by activator protein 1 (AP1) and CCAAT/enhancer-binding protein (CEBP) following stimulation of the ROCK and the p38MAPK pathways (Fig. 3) (Cattaruzza et al., 2001).

Lung

Embryonic lung development is highly sensitive to mechanical cues (Costa et al., 2001; Mendelson, 2000; Minoo, 2000). Before birth, intraluminal pressures are generated by secretion of fluid by the pulmonary epithelium and the elastic recoil properties of the lung tissues (Harding et al., 1986; Vilos and Liggins, 1982). Physical stretching of lung epithelial cells activates NF-κB (Harding and Hooper, 1996), which enhances maturation of lung epithelial cells during embryogenesis in mouse (Londhe et al., 2008). In chicken lung, NF-κB activity in the mesenchyme also has a role during morphogenesis of lung branching (Muraoka et al., 2000). Similarly, the level of tensile prestress (isometric tension) generated by Rho-dependent changes in cytoskeletal contractility affects lung branching morphogenesis in mice (Moore et al., 2002; Moore et al., 2005). Mechanical stretching of the lung also drives myogenesis in airway smooth muscle, which is crucial for bronchial development. This process is mediated by the activation of the serum response factor (SRF) transcription factor (Badri et al., 2008; Yang et al., 2000). Overdistention of the fetal lung (as seen, for example, in congenital laryngeal atresia) results in the formation of larger lungs with an increased number of alveoli and a more mature architecture than expected for gestational age in humans (Wigglesworth et al., 1987). During the transition to breathing air at birth, the lung also undergoes dynamic physical changes that appear to influence formation and maturation of alveolar structures (Inanlou et al., 2005; Nelson et al., 2005; Wigglesworth et al., 1987). For example, in vitro studies have demonstrated that differentiation of rat embryonic lung cells into either type I or II alveolar cells, is modulated by mechanically distending cells in a way that mimics inflation and deflation of the alveolus during respiration (Gutierrez et al., 1999; Wang et al., 2006).

The gastrointestinal and musculoskeletal systems

Tissues within many other organs have been shown to exhibit mechanosensitive transcriptional regulation. For instance, mechanical pressure in the gut stimulates gene transcription and proliferation in rat gastrointestinal epithelial cells through MAPK-mediated activation of the transcription factor AP1 (Hirokawa et al., 2001). Exposure of these cells to pressure also modulates the synthesis of interleukin 6 (IL6) through the NF-κB pathway, which is crucial for gut homeostasis (Kishikawa et al., 2002). Mechanical loading, generated by gravity or physical movement, similarly stimulates the formation of bones, joints, tendons, ligaments, cartilage and muscle within the musculoskeletal system in humans and rats (Kahn et al., 2009; Lanyon, 1992; Raab-Cullen et al., 1994; Robling et al., 2006; Rubin and Lanyon, 1985). Many of these effects are mediated through the activation of specific transcription factors. For example, mechanical deformation of osteoblasts activates MAPK, which binds to and phosphorylates the transcription factor RUNX2, which subsequently controls expression of osteoblast-specific differentiation genes, including osteocalcin, type I collagen, bone sialoprotein, osteopontin and alkaline phosphatase in humans and rodents (Kanno et al., 2007; Ziros et al., 2008).

Similarly, during differentiation of skeletal muscle, the expression of specific sets of genes leads to the production of myofibril proteins and their assembly into contractile sarcomere units that are constantly remodeled in response to changes in mechanical load. Calcineurin, a molecular target of intracellular Ca^{2+}–calmodulin signaling activates the transcription factors myocyte enhancer factor 2 (MEF2) and myogenic differentiation

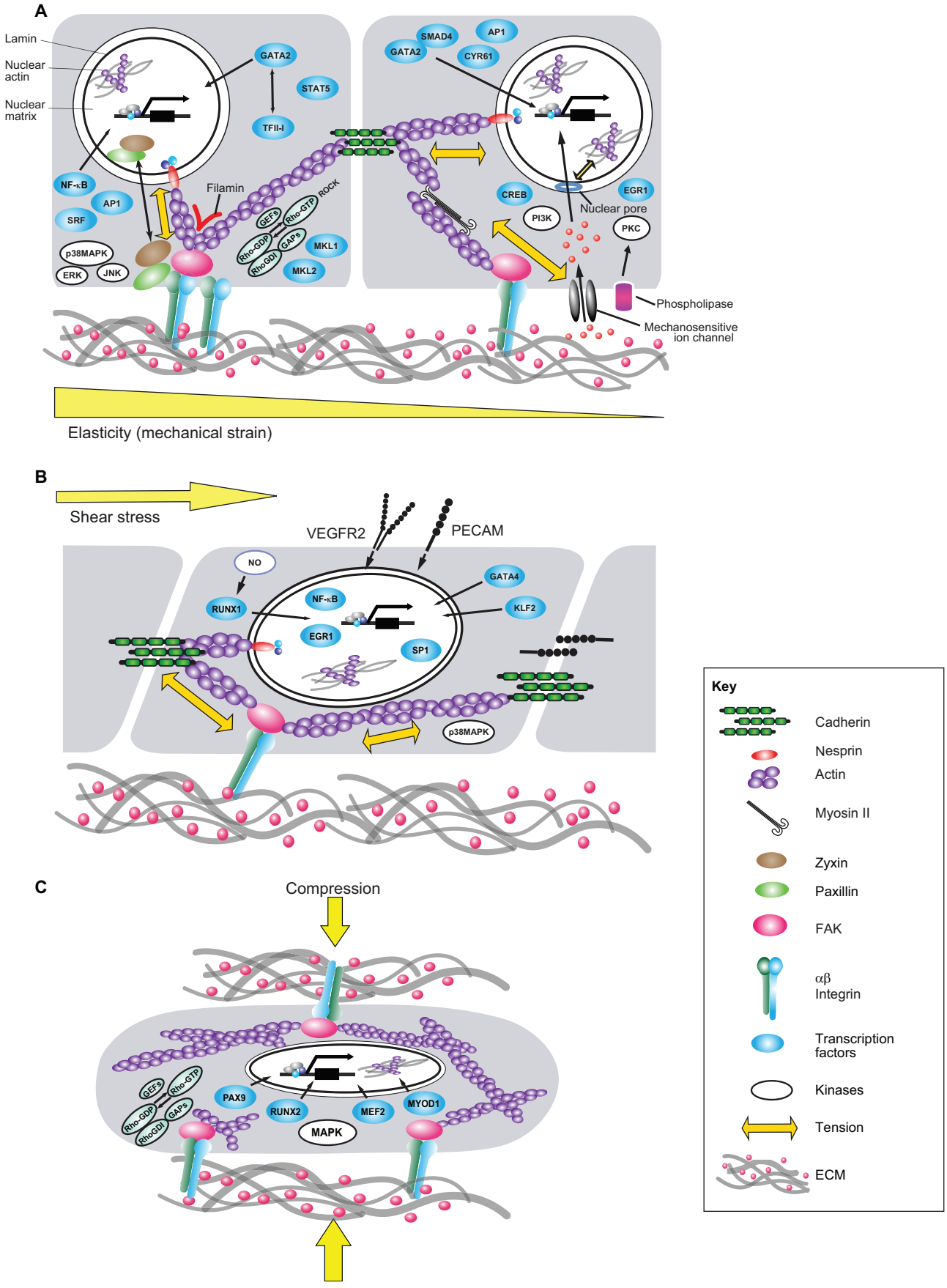

Fig. 3. See next page for legend.

1 (MYOD1), which mediate mechanical-load-induced myogenesis by inducing the expression of myogenin in mice (Friday et al., 2000; Friday et al., 2003; Parsons et al., 2004). Mechanosensitive endocytosis of BMP2 also has a crucial role in the transcriptional control of myogenic and osteoblastic differentiation in mouse mesenchymal stem cells (Rauch et al., 2002).

Deregulation of mechanosensitive transcriptional control and disease development

It is important to note that deregulation of normal mechanosensitive mechanisms of transcriptional control can lead to pathological changes (Table 1). In rodents, for example, excessive hemodynamic loading results in cardiac hypertrophy through exaggerated transcriptional activation of smooth muscle α-actin (ACTA2) (Mack and Owens, 1999; Zhao et al., 2007). The exposure of tissues to excessive mechanical forces increases the expression of pro-inflammatory proteins, such as interleukin-1β (IL1B), tumor necrosis factor α (TNFA), inducible oxide synthase (iNOS, also known as NOS2) and matrix metalloproteinases. Continued activation of these responses can lead to inflammatory diseases, such as arthritis (Agarwal et al., 2004; Ray et al., 2005; Yang et al., 2005), asthma (Kumar et al., 2003) and ventilator-induced lung injury (Ning and Wang, 2007).

At the same time, sustained application of mechanical force can sometimes repair diseased tissues. For instance, in a process referred to as distraction osteogenesis, gradual traction on bones is used to enhance osteogenesis for the treatment of skeletal deformities in dogs and humans (Ilizarov, 1989a; Ilizarov, 1989b; Aarnes et al., 2002). Similarly, constant stretching of the intestines leads to an increase in intestinal length in rats (Park et al., 2004) and stretching induces regeneration and lengthening of the esophagus in human infants with esophageal atresia (Foker et al., 1997). However, little is known about the transcriptional processes that mediate these mechanoresponses.

Fig. 3. Mechanotransduction pathways and transcriptional control in response to mechanical strain, fluid shear stress and compression. Cells and tissues are stabilized through a tensegrity mechanism in which a stabilizing tensional prestress or state of isometric tension is generated inside the cells by mechanical forces. The latter are generated by interactions of actin and myosin within the cytoskeleton that are worked against by integrin adhering to the ECM, cadherin adhering to neighboring cells and by internal cytoskeletal structures (Ingber, 2006). This mechanical equilibrium governs cell mechanics and hence, modulates the response of cells to external forces. (**A**) When cells and tissues experience mechanical strain, forces transmitted through integrin receptors and cadherins alter intracellular signaling pathways involving proteins such as kinases (e.g. PKC, MAPK14, ERKs and JNK), focal adhesion proteins (e.g. FAK, zyxin, paxillin) and Rho small GTPases and its guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). Mechanical tension exerted on integrins also modulates mechanosensitive ion channels and phospholipases, which activate PI3K and PKC, respectively, through second messengers such as intracellular Ca^{2+}, inositol lipids and arachidonic acid. All of these mechanochemical signals lead to the activation of transcription factors (e.g. GATA2, TFII-I, NF-κB, AP1, SRF, STAT5, MKL1, MKL2, SMAD4, CYR61, CREB and EGR1). Physical forces that are exerted on surface adhesion receptors and are transmitted directly to the nucleus along cytoskeletal filaments and molecules that connect the cytoskeleton to the nucleus, such as nesprin, can influence transcription activity more directly. (**B**) When adherent cells, such as endothelial cells, are exposed to apical fluid shear stress, the cells sense these forces through mechanosensors such as VEGFR2 or PECAM. Various transcriptional activities (e.g. RUNX1, GATA4, KLF2, NF-κB, EGR1 and SP1) are modulated through downstream changes in biochemical signaling (e.g. NO, p38MAPK) and cytoskeletal prestress. (**C**) Compressive forces generated by gravity or physical movements that are transmitted across the ECM can also alter activities of intracellular biochemical signaling molecules (e.g. Rho GTPases, their GEFs and GAPs and MAPK) and cytoskeletal tension. Again, these changes can modulate various transcriptional activities (involving for example PAX9, RUNX2, MEF2 and MYOD1) that are crucial for homeostasis of tissues that normally bear compressive forces, such as bone, cartilage and tooth. Parts of this figure were modified with permission from Mammoto and Ingber, 2010 (Mammoto and Ingber, 2010).

Mechanotransduction and transcriptional control

The multiple examples described above clearly demonstrate that mechanical forces can regulate the activity of transcription factors and the expression of genes that are crucial for developmental control and tissue maintenance. By contrast, the varied mechanisms through which mechanical forces applied at the cell surface or generated within the contractile cytoskeleton can produce these transcriptional alterations have only come to light recently.

Mechanosensitive transmembrane receptors

Mechanical forces can alter transcription by activating transmembrane receptors and harnessing intracellular signaling cascades that are used by soluble hormones and cytokines to alter the transcription factor activity. Work over the past two decades has revealed that mechanical signals are preferentially sensed by transmembrane surface adhesion receptors, such as integrins and cadherins, which can transfer physical forces across the plasma membrane (Fig. 3). Such mechanical signals are converted into cytoplasmic biochemical signals within cell surface focal adhesions and cell–cell adhesion complexes (reviewed in Mammoto and Ingber, 2010). These adhesion complexes can trigger transcriptional changes in the nucleus through modulating downstream signaling cascades (Ingber, 2006; Mammoto and Ingber, 2009; Mammoto et al., 2008; Orr et al., 2006). Mechanical signals can, thereby, elicit changes in the concentration of second messengers, such as Ca^{2+} flux, inositol trisphosphate ($Ins P_3$), diacylglycerol (DAG), cAMP and nitric oxide, or activate protein signaling molecules, such as MAPKs and Rho-family small GTPases, which are involved in controlling virtually all cellular processes, including migration, growth, differentiation and matrix remodeling (Alenghat et al., 2009; Balligand et al., 2009; Huang and Ingber, 2002; Mammoto and Ingber, 2009; Mammoto et al., 2008; Martinac, 2004; Orr et al., 2006; Papachristou et al., 2009; Sadoshima and Izumo, 1993).

One of the most rapid mechanical signaling pathways upstream of transcription involves the activation of mechanosensitive ion channels on the cell surface. In endothelial cells, cyclic mechanical strain applied through the ECM and ECM-bound integrin receptors induces Ca^{2+} influx through mechanosensitive transient receptor potential cation channel subfamily V member 4 (TRPV4) channels within 5 mseconds after the application of the mechanical force (Matthews et al., 2010). The resultant change in membrane potential stimulates phosphatidyl inositol-3-kinase (PI3K), which activates additional β1 integrin receptors and promotes cytoskeletal remodeling that is crucial for angiogenesis (Fig. 3) (Thodeti et al., 2009). In neurons, Ca^{2+} flux through mechanosensitive ion channels activates the ERK1 and ERK2 (ERK1/2, also known as MAPK3 and MAPK1, respectively) pathway and stimulates the expression of genes that are essential

Table 1. Deregulated mechanotranscription in different disease-states

Organ system	Mechanical deregulation	Pathological conditions	Transcriptional regulation	References
Heart	High pressure and/or volume	Cardiac hypertrophy	MKL1[a]	(Zhao et al., 2007)
Blood vessel	Turbulent flow	Atherosclerosis	NF-κB[b], EGR1[b]	(Gimbrone et al., 2000)
Lung	High pressure	Asthma	NF-κB[b]	(Kumar et al., 2003)
Lung	High pressure	Ventilator-induced lung injury	NF-κB[b]	(Ning and Wang, 2007)
Gastrointestinal tract	High pressure	Irritable bowel syndrome	AP1[b]	(Hirokawa et al., 2001)
Bone	Low pressure	Osteoporosis	FOS[b]	(Bergmann et al., 2010)
Joint	High pressure	Arthritis	NF-κB[b]	(Agarwal et al., 2004)
Muscle	Low pressure	Muscle atrophy	FBXO32[b], MURF1[b]	(Sandri, 2008)

[a], co-activator; [b], transcription factor.

for neuronal survival, plasticity and memory formation through activation of the transcription factor cAMP responsive element binding protein (CREB) (Dolmetsch et al., 2001; Wheeler et al., 2008).

Mechanical forces activate MAPK pathways including the ERK1/2, p38 MAPK (also known as MAPK14) and Jun N-terminal kinase (JNK, also known as MAPK8) pathways in endothelial cells, osteoblasts and fibroblasts by stimulating focal adhesion kinase (FAK) in focal adhesions (Boutahar et al., 2004; D'Addario et al., 2002; Huang and Ingber, 2002; Ishida et al., 1996). Mechanically activated ERK1/2 and/or JNK signals are transmitted into the nucleus where they activate the transcription factor AP1 and, thereby, upregulate expression of molecules that are crucial for tissue formation and remodeling, such as type I collagen and osteopontin in bone (Hong et al., 2010; Jeon et al., 2009; Kook et al., 2009). Mechanical strain and distortion of the cell shape also activate membrane-associated phospholipases and, thereby, increase the metabolism of inositol lipids and arachidonic acid in the cytoplasm, which results in the release of Ca^{2+} from intracellular stores, activation of protein kinase C (PKC) and the remodeling of cardiomyocytes through activation of mechanosensitive transcription factors, such as EGR1 and AP1 (Fig. 3) (Bishop and Lindahl, 1999; Komuro et al., 1991; Sadoshima and Izumo, 1993; Tseng et al., 1994). These findings suggest that mechanical cues are transmitted to the nucleus through an interplay between mechanosensitive transmembrane receptors and a variety of second messengers that modulate transcriptional activities that are crucial for cell differentiation and tissue remodeling.

The cytoskeleton as a regulator of transcription

Cells and tissues that are tensionally prestressed are held in a state of isometric tension as a result of micromechanical contractile forces, which are generated by ECM adhesions as well as adhesive contacts to neighboring cells that balance the cytoskeletal actomyosin filaments. These forces, in turn, stabilize cell shape and mechanics through a tensional integrity ('tensegrity') mechanism (Ingber, 1993). Mechanotransduction is sensitive to this micromechanical level of prestress in the cells, which is stored and released to control mechanotransduction in a way that resembles the use of a bow and bow string (Ingber, 2006).

Focal adhesion proteins that physically couple actin filaments to integrins and mediate transmembrane mechanical force transmission, including talin, paxillin, filamin A and vinculin, also act as mechanotransducers, in part by regulating cytoskeletal tension (Chan et al., 2009; Riveline et al., 2001). Part of this response is mediated by regulating the physical strength of the focal adhesion that resists cell traction forces to sustain cytoskeletal prestress (Fig. 3) (Ezzell et al., 1997). These focal adhesion proteins also facilitate translation of mechanical cues into chemical signals that modulate the Rho signaling pathway, which feeds back to control phosphorylation of myosin II and the generation of cytoskeletal tension (Mammoto et al., 2007; Zhao et al., 2007). Moreover, some focal adhesion proteins, such as paxillin and zyxin, alter their binding kinetics in a force-dependent manner (Lele et al., 2006). This enables them to shuttle between the cytoplasm and nucleus where they can act directly as co-activators of transcription to regulate gene expression (Wang and Gilmore, 2003).

In addition to the involvement of various signaling and adhesion proteins, the actin cytoskeleton itself seems to have a central role in coupling mechanical forces to the regulation of tissue growth at a transcriptional level (Wang and Riechmann, 2007). For example, actin cytoskeleton-dependent control of Rho GTPase activity through p190RhoGAP (also known as ARHGAP35) mediates cell-shape-dependent changes in cell-cycle progression by altering the balance between ROCK and mammalian Diaphanous-related formins (mDia) (Mammoto et al., 2007; Mammoto et al., 2004). When the actin cytoskeleton is disrupted and cells are round, filamin A binds to p190RhoGAP and its activity as a GTPase activating protein (GAP) is inhibited, leading to the activation of Rho (Mammoto et al., 2007). By contrast, in spreading cells, filamin A, an actin-binding protein that crosslinks F-actin, is cleaved by calpain and p190RhoGAP dissociates from filamin A. Subsequently, RhoGAP moves to the lipid-raft fraction where it inactivates Rho (Mammoto et al., 2007). This distortion-dependent signaling mechanism mediates cell-shape-dependent changes in cell cycle progression (Mammoto et al., 2004; Mammoto et al., 2007). Myocardin-like proteins 1 and 2 (MKL1 and MKL2, respectively), which are co-activators of SRF, also have a key role in controlling Rho-dependent gene expression and cell growth (Descot et al., 2008). RhoA regulates the nuclear translocation of MKL1 and/or MKL2 and mediates the formation of stress fibers and focal adhesions by activating the transcription of cytoskeletal and/or focal adhesion genes (Morita et al., 2007).

Human mesenchymal stem cells (MSCs) express organ-specific transcription factors and undergo tissue-specific cell fate switches (e.g. neuron vs myocyte vs osteocyte) when cultured on ECMs with mechanical stiffnesses that mirror the physical properties (i.e. the Young's moduli) of their respective tissues (e.g. brain vs muscle vs bone) (Engler et al., 2006). Changes in ECM compliance alter cytoskeletal tension, actin

structure and cell shape (Ingber, 2003; Polte et al., 2004), and the effects of ECM compliance and cell shape on MSC fate switching is mediated by Rho and Myo-II, which generate cytoskeletal tension (Fig. 3) (Engler et al., 2004; Ren et al., 2009). These findings further suggest that the actin cytoskeleton and tensional prestress are central mechanosensitive control elements, which are involved in a complex feedback loop: they both mediate the effects of mechanical cues on transcription regulation and alter their functional state in response to changes in gene transcription.

The nucleus as a mechanical point of control of transcription

The cytoskeleton is coupled to the nuclear membrane, intranuclear scaffolds and chromatin, and molecular connections between integrins, cytoskeletal filaments as well as nuclear scaffolds provide a discrete path for the transmission of mechanical signals in response to changes in adhesion to the ECM or in the cytoskeletal prestress (Maniotis et al., 1997b) (Fig. 3). In fact, changes in nuclear mechanics and architecture that are modulated by the ECM substrate, focal adhesions and the cytoskeleton, have crucial roles in the transcriptional control of cell fate determination (Buxboim et al., 2010). Importantly, changes in cell fate between growth, differentiation and apoptosis can be controlled by altering cell shape (Chen et al., 1997; Dike et al., 1999), ECM elasticity (Engler et al., 2006), cytoskeletal contractility through Rho–ROCK signaling (McBeath et al., 2004) and tissue patterns (Ruiz and Chen, 2008). Given that nuclear mechanics are regulated by these same parameters (Khatau et al., 2010; Kihara et al., 2011; Sims et al., 1992), the nucleus itself might act as a mechanosensor that directly modulates regulation of transcription during cell fate determination in these cells. In support of this possibility, inhibition of Myo-II with blebbistatin or disruption of actin microfilaments with cytochalasin D, results in a dramatic decrease in the nuclear size of adherent cells, whereas inhibition of microtubule polymerization with nocodazole increases the nuclear size (Mazumder and Shivashankar, 2010). Thus, the area of the cell surface and the size of nuclei are directly coupled to integrin through cytoskeletal linkages; this physical connection results in the nucleus becoming prestressed, so that it balances contractile forces of the cytoskeleton with condensation forces of chromatin (Mazumder and Shivashankar, 2007; Mazumder et al., 2008; Mazumder and Shivashankar, 2010). Importantly, such a mechanism of nuclear mechanotransduction in response to forces that act on the cell surface is much faster than the propagation of signaling ions and molecules (Li et al., 2007). Nuclear prestress might also be crucial to determine the cell fate (Fig. 3), because stem cell differentiation leads to the dynamic reorganization of connections between the cytoskeleton and the nuclear membrane, thereby progressively leading to a stiffer nucleus as lineage-committed cells emerge (Mazumder and Shivashankar, 2010; Pajerowski et al., 2007).

Interestingly, the structure of the nucleus also appears to be crucial for transcriptional reprogramming of pluripotency. For example, transplantation of somatic nuclei into eggs or oocytes is sufficient to reactivate silenced pluripotent genes such as that encoding OCT4 (*Pou5f1*) (Kim et al., 2009; Stadtfeld et al., 2008). Moreover, this is a more-efficient way to reprogram cells into pluripotent stem cells than the forced induction of OCT4, SOX2, MYC and KLF4 protein expression in somatic cells (Kim et al., 2010; Pasque et al., 2010). Although the mechanism by which the physical structure of the nucleus induces gene reprogramming at the level of transcription is unclear, it is known that nuclear actin can regulate gene transcription through changes in cytoskeletal actin dynamics (Sotiropoulos et al., 1999) or by modulating the assembly of transcriptional regulatory complexes in the nucleus (Grummt, 2006). Nuclear actin polymerization also has an essential role in transcriptional reactivation of the gene encoding OCT4 in transplanted nuclei (Miyamoto et al., 2011), and decreased cytoskeletal tension destabilizes transcriptional regulation of pluripotency and, hence, compromises long-term survival of ESCs (Li et al., 2010). The different effects of pluripotency on transcriptional control in ESCs might, therefore, be regulated by mechanical forces that are exerted on the cell surface and transmitted to the nucleus across transmembrane adhesion receptors (e.g. integrins and cadherins) and the cytoskeletal filaments associated with these receptors, which result in tension-dependent changes in nuclear structure (Maniotis et al., 1997a; Maniotis et al., 1997b; Sims et al., 1992; Wang et al., 1993; Wang et al., 2009).

Mechanical forces can also regulate gene expression directly by altering the transport of transcription factors or other molecules into the nucleus. For example, nuclear transport of MKL1 is regulated by prestress in fibroblasts (McGee et al., 2011). Nuclear transport of NF-κB, which activates expression of the genes encoding PDGFA and PDGFB in response to fluid shear stress, is also specifically sensitive to mechanical perturbation in endothelial cells (Khachigian et al., 1995; Meiler et al., 2002). The levels of nuclear translocation of the two transcription factors TFII-I and GATA2, which control angiogenesis by regulating the expression of VEGFR2, have been shown to be sensitive to ECM mechanics in endothelial cells (Mammoto et al., 2009). In spreading cells, nuclear membrane distortion – as a result of cytoskeletal distortion – also stimulates cytoskeletal tension-dependent Ca^{2+} entry through nuclear ion channels (Fig. 3) (Prat and Cantiello, 1996) and induces gene transcription (Itano et al., 2003). Because nuclear envelope proteins, such as lamin A and emerin, bind to transcription factors and because these proteins are directly linked to the cytoskeleton through nesprin (Mejat and Méjat, 2010; Wang et al., 2009), any forces that are transferred through these molecules might also alter gene expression directly (Dreuillet et al., 2002; Haraguchi et al., 2004).

Taken together, these findings suggest that the micromechanical environment of the nucleus modulates the transcriptional activities that are crucial to control cell fate determination, and that are sensitive to the microenvironment of living embryonic and adult tissues. Furthering our understanding of these mechanisms will also be crucial for the development of new strategies to reprogram cells and to treat congenital diseases that are caused by perturbed nuclear structure and mechanics, such as Hutchinson–Gilford progeria syndrome, Emery–Dreifuss muscular dystrophy, dilated cardiomyopathy and familial partial lipodystrophy (Worman et al., 2009).

Conclusions

In this Commentary, we have reviewed multiple findings that demonstrate the central role of mechanosensitive control mechanisms of transcription that determine stem cell fate, embryogenesis and that control organ homeostasis. It is now

clear that both external stresses and intracellular cytoskeletal tension can be driving forces behind changes in transcriptional regulation. These mechanical signals influence transcription by altering intracellular signaling pathways or mediating changes in cytoskeletal and nuclear structure or mechanics. The extent of this response and the efficiency with which the mechanical signal is transferred through the cell and to the nucleus depend on the level of isometric tension or prestress in the cytoskeleton. Cell-generated tensile forces not only alter the chemical signals that are conveyed by cells, but also produce distortion of neighboring cells and ECM molecules that propagate mechano-chemical signaling over long distances. This process drives tissue patterning and organ formation on the embryonic and tissue scale. These findings also raise the possibility that a better understanding of these mechanotranscriptional control mechanisms will increase our chance of reversing developmental defects or treating diseases by restoring normal mechanical loading conditions or correcting abnormal transcriptional activities.

Despite a great effort to engineer 3D organs from ESCs, so far this approach has not been fully successful because of the lack of a comprehensive understanding about how tissues develop regarding a specific size, shape and material property during organogenesis. The necessity to integrate mechanosensitivity into existing models of transcriptional control to fully understand tissue and organ development is becoming clear. Advances in this area will require new methods in order to measure endogenous mechanical forces and local variations of the mechanical properties of cells, the ECM and entire tissues within forming organs, and a way to analyze how mechanical cues and chemical signals dynamically interact to regulate transcriptional activities during important morphogenetic and patterning events. Because cellular mechanotransduction is central to the control of developmental processes and tissue homeostasis, a deeper understanding of mechanosensitive transcriptional control mechanisms in embryonic and adult organs might also lead to new forms of therapeutic intervention for various diseases, as well as new strategies for tissue engineering and regenerative medicine.

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