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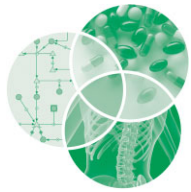

Mechanotransduction: a major regulator of homeostasis and development

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In nearly all aspects of biology, forces are a relevant regulator of life's form and function. More recently, science has established that cells are exquisitely sensitive to forces of varying magnitudes and time scales, and they convert mechanical stimuli into a chemical response. This phenomenon, termed mechanotransduction, is an integral part of cellular physiology and has a profound impact on the development of the organism. Furthermore, malfunctioning mechanical properties or mechanotransduction often leads to pathology of the organism. In this review, we describe mechanotransduction and the theories underlying how forces may be sensed, from the molecular to organism scale. The influence of mechanotransduction on normal and abnormal development, such as stem cell differentiation and cancer, is also reviewed. Studies illustrate the diversity of mechanotransduction, and the major role it has on organism homeostasis. Cells employ a variety of mechanisms, which differ depending upon cell type and environment, to sense and respond to forces. © 2010 John Wiley & Sons, Inc. *WIREs Syst Biol Med*

In general, all animals consist of four general tissue types: nervous, skeletal, connective, and epithelial tissues. These tissues are organized into distinct geometries and hence underlie the functional aspects of many organs and organ systems. To survive, an organism relies on the proper functions of organs and each organ in a body satisfies a specific survival demand, such as bodily fluid waste removal (kidneys), nutrient and oxygen perfusion to tissues (heart), absorption and solid excretion (gastrointestinal tract), and gas-exchange (lungs).

The four tissue types can be thought of as having general roles for each organ. Nervous tissue, for instance, is responsible for the conduction of signals to the organ, and can toggle organ function on or off. Connective tissues form the scaffolding of the organ and are responsible for forming the matrix of the organ's cells and for the stable anchoring of the organ in the animal. Physical forces are provided by muscular tissue. Muscular tissue can force large-scale transport of substances into and out of an organ, but can also support tissue architecture. The fourth tissue type, epithelial tissue, can be thought of as the tissue underlying absorption, excretion, secretion, sensation, and protection.

Although these general concepts can be applied to organs, not all organs necessarily contain all four tissue types. Evolution has specialized many organs to carry out specific tasks, such as the central nervous system. The central nervous system can be considered to contain mainly nervous tissue and very little, if any, muscular tissue. In addition, these tissue types are intimately associated and rely upon one another for function.

In contemporary medicine, organs are thought of as modular in structure and function. However, organs work as systems and cooperatively in physiology. For example, waste removal requires not only the kidneys for filtration of bodily fluids, but also the action of the liver to prepare compounds for excretion. To function properly, the kidneys also require the action of the perfusion system, driven by the heart. The heart is, in turn, dependent upon the respiratory system, consisting of the lungs and breathing muscles for its oxygen demand.

Recent advancements in molecular and cellular biology allowed the discovery that tissue development

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is not absolutely autonomous, but tissues of a developing organism coordinate their maturation in an integrated manner.[1,2] These studies are astounding in that they implicate that many tissues of different fates and origins communicate with one another reciprocally.

Another landmark of the 21st century science is the discovery that cells and tissues communicate and respond to forces.[3–6] The transduction of mechanical stimuli into a cellular signal and response is termed mechanotransduction.[3–7]

To achieve homeostasis, feedback exists between the targets of signaling and the origin of the signal. There is thus reciprocity in signaling pathways. The target reciprocally communicates with the originator of the signal to modulate future responses. Reciprocity of signaling during development is not limited to electrochemical signals, but can also occur through mechanical means, and this can be termed mechanoreciprocity.[8,9] Developing tissues can thus interact via forces and through physical contact. Thus the aim is to understand both the biological and the mechanical interactions of cells and tissues. By understanding how tissues interact to form biological organs of the developing organism, one can hopefully exploit these in regenerative medicine, for example.

Mechanotransduction research is fundamental in the underlying concepts of being able to one day guide cells spatially and temporally to create defined structures in three dimensions. Cells *in vivo* are constantly reshaping tissues by varying mechanical and biochemical properties, both spatially and temporally.[10,11] These changes, on a grander scale, ultimately result in what is termed morphogenesis, differentiation, determination, and are a part of development.

Major developmental processes are influenced by mechanotransduction. For instance, what marks the left and right sides of the organism is determined by coordinated ciliary beating which is thought to influence local fluid dynamics in the cellular environment.[12] In addition, morphogenesis of organs often involves a complex execution of forces to result in proper tissue topology and shaping, such as during the formation of the chambers of the heart.[13,14]

Additionally, mechanotransduction can underlie many abnormal processes in organisms. For example, one of the first diseases to be correlated with biomechanics and mechanotransduction is atherosclerosis, a disease that is now the leading cause of death in the United States.[15,16] Researchers have shown that low-oscillatory shear stress correlates with sites of atherosclerotic plaques, and that the low-shear stress environment dramatically alters endothelial organizations, especially their cytoskeleton.[17–20]

For centuries, orthopedists have known that bone growth and healing, among the many factors including diet, are also correlated to weight bearing activities. Since then, many other examples of how mechanics plays a major role in biology have surfaced, but how this process specifically occurs at the molecular and cellular levels remains elusive.

In the remainder, we will review the concepts that define the field of mechanotransduction beginning with the molecular scale and proceeding to the organ scale. We discuss the current theories as to how molecules can be influenced by force and the corresponding reactions of the cells and tissues that sense these changes. The force and time scales that life is sensitive to are also relevant to understanding mechanotransduction theory. Lastly, we discuss how mechanotransduction plays a role in normal and abnormal developmental processes and propose some areas of inquiry for future study.

MOLECULAR CELL BIOMECHANICS

Ultimately, intracellular propagations of all stimuli occur through biochemical cascades to alter transcription and cellular activity. Until the origin of mechanotransduction theory, it was widely assumed that chemically propagating stimuli also began through biochemistry, i.e., binding and oligomerization or changes in electrical conductance leading to binding or enzymatic conformational changes. However, recent evidence has led to the discovery that cells also respond to their physical environment and these are transduced into intracellular biochemical cascades. Therefore, the term 'mechanotransduction' describes the specific capacity of life to transduce a mechanical signal into a biochemical signal. Mechanotransduction is a rapidly growing field since its implication in the mid-1960s by Y.C. Fung.[7,21]

Interestingly, although it is known that mechanical factors do dramatically alter cellular and even tissue behaviors, their relationship to biochemical signals remains elusive. Even further, it is debated whether or not cells are actually actively responding to the mechanical factors, or if the mechanical factors passively enable other cell behaviors. For example, the observation that cells migrate and behave more mesenchymal-like on stiffer substrates may not be that cells actually respond to the stiff substrate, but rather that certain expression patterns are enabled in the presence of greater physical substrate support.[22] A cell's intention is difficult, if not impossible, to justify. Nevertheless, mechanics cannot be neglected if one

were to try to understand cellular development and function.

Forces are increasingly being recognized as major regulators of cell structure and function.[23] The balance of forces among cells determines multicellular organization as much as the expression of genes does. Recent studies *in vitro* and even in model organisms, such as *Drosophila melanogaster* or *Caenorhabditis elegans,* combine physical modeling with experimental measurements to provide insight on the processes that influence cell patterns *in vivo*.[24]

MECHANOTRANSDUCTION

A major hallmark of modern molecular and cell biology is the discovery of the cytoskeleton in both prokaryotic and eukaryotic organisms. This discovery prompted the revision of the model that cells are basically bags of enzymes. The cytoskeleton is a stress bearing structure and governs cell shape. Structurally, the cytoskeleton also serves as the mechanical link between the exterior and internal environments.

During processes like cell movement or shape change, the cytoskeleton is actively and dynamically remodeling. The precise regulation of cell movement is the epitome of mechanotransduction. For example, a cell that is migrating must be able to transduce an initial chemical cascade into a physical effect. Furthermore, the cell must also be able to recognize what forces it has applied and how the cytoskeleton is organized in order to successfully carry out its movement. Thus, a cell must in every day activity be able to both transduce a biochemical stimulus into a mechanical effect, but also vice-versa, transduce a mechanical stimulus into a biochemical effect, the latter we typically call mechanotransduction.

It seems obvious to some degree that the former case is entirely possible through the action of force producing enzymes, such as motor proteins and cytoskeletal filament polymerizations. However, how the cell can recognize the force it is applying and the force it feels from the external environment is not so clear. Biochemical pathways in the cell are under precise regulation, require an input of energy, and are not generally reversible. These two pathways, although seemingly related, proceed via unique mechanisms.

Recently, the cytoskeleton has also been implicated in serving as a scaffold for many biochemical activities. Transmembrane chemoreceptors or channels often link to the cytoskeleton, namely actin.[25,26] Even classical signal transduction cascades are often demonstrated to be intimately linked and localized to the cytoskeleton.[25,26] In fact, some normal and pathogenic processes rely on their anchors to the cytoskeleton to carry out their activities. For example, the human immunodeficiency virus requires cytoskeletal binding to endocytose and infects cells.[27] One can readily identify, therefore, that mechanics plays a large role in biological behaviors at varying scales, from the molecular level to the scale of an organism.

One must take into account that the magnitudes of length and time at the molecular scale may difer from the macroscopic scale. The challenge of modern biomechanics and mechanotransduction is to bridge the gap between the micro and macro scales. This will depend largely upon the integration of knowledge from the molecular to the organism scale. Ideally, one would be able to one day extrapolate from biomechanics at the molecular scale and predict the macroscopic effects at not only the cell scale, but also the tissue, organ, and even on the level of the whole organism (Figure 1). For example, when a gene encoding an extracellular component protein in *D. melanogaster*, *bola,* is mutated, the egg chamber of the organism develops spherically rather than oblong in wild type[28–31] (Figure 1). Although multiple mutations in differing extracellular matrix (ECM) encoding genes result in similar phenotypes, the mechanical implications of such mutations at the cellular scale underlying the phenotype, nor the tissue morphogenetic alterations are known.[28–31]

Theoretically, one should be able to identify the cellular changes, which could underlie changes in the tissue morphology (Figure 1). Although these assertions are highly speculative and the goal ambitious, the use of such knowledge could be useful for diagnosing the molecular bases of diseases and future cures. Such foresight could also be used in designing future tissues and organs for regenerative medicine.

MECHANOTRANSDUCTION INFLUENCES PHYSIOLOGY

The cardiovascular system is markedly sensitive to mechanical stress. It is well established that fluid flow forces regulate the development and physiology of the heart and the vascular network.[32–36] At the molecular and cellular scale, vascular endothelia align their F-actin stress fibers along the primary orientation of fluid shear stress.[37] In areas of high unidirectional oscillatory shear stress, vascular endothelium exhibits a uniform orientation of their cytoskeletal network, which produces a cylindrical supracellular arrangement, and is atheroprotective.[38,39]

In areas of low oscillatory shear stress or recirculation zones, the vascular endothelium develops a random orientation of stress fibers and does not

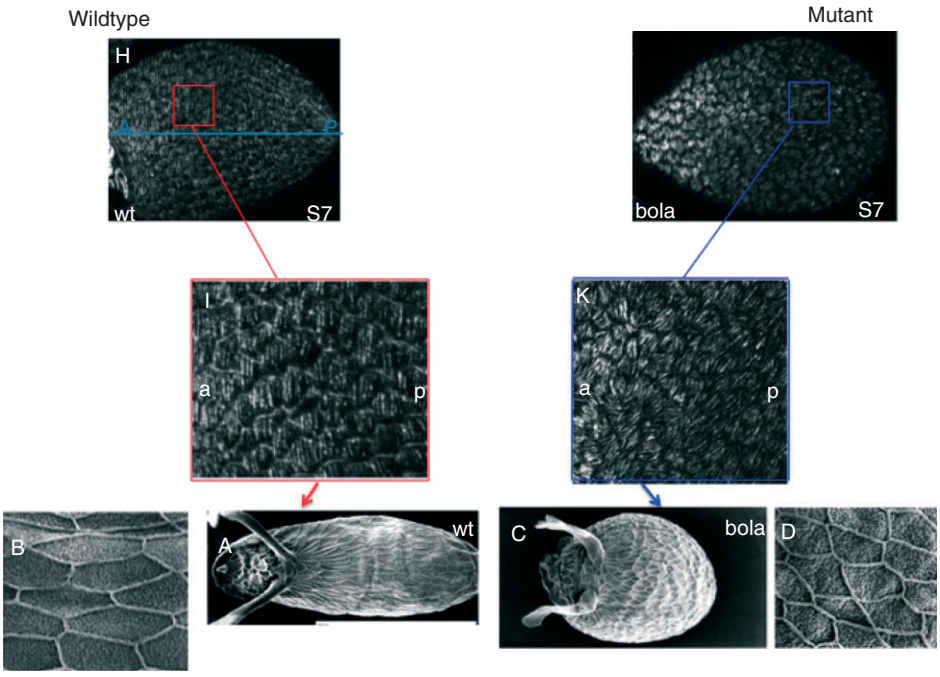

FIGURE 1 | A single mutation in a gene dramatically alters the local cell shape and also the global tissue morphology. The organism develops abnormally spherical. In normal development the epithelium appears stretched a long a uniform axis, and the mutation disrupts this process. The mutation results in a decrease in net force within the epithelium. (Adapted with permission from Ref 30. Copyright 2001 Development).

form a supracellular structure. At these sites, there is a greater propensity for vascular wall thinning, resulting in aneurysms, or atherosclerotic plaques.[39–42] Some researchers speculate that wall thinning may be a homeostatic response to increase tissue stresses in the endothelial wall.

Another example where fluid shear stresses influence tissue homeostasis is in bone. In haversian or cortical bone, osteocytes are thought to regulate bone structure by responding to dynamic fluid shear stresses that are normally generated during movement.[43]

Bone cells, osteocytes, extend long membranous processes through bone channels, canaliculi, and connect to share cytoplasmic material via gap junctions. The osteocytes can thus respond to fluid flow that is induced by squeezing during bending of stress in bone.[44]

THE MOLECULAR BASIS OF MECHANOTRANSDUCTION

The signaling cascades that become activated by mechanical stress are actively studied. However, the means by which this mechanical stress is initially converted into a biochemical signal is much less understood. A myriad of theories exist that might explain the process of mechanotransduction, but most require further development and refinement.

An excellent example of mechanotransduction is the sensory system of hair follicles. In hair follicles, the cilia that compose the hair bundle are physically linked to transmembrane potassium channels.[45] Any force that is applied to the hair deflects the cilia and in turn stretches the potassium channels and the potassium influx initiates the biochemical-signaling cascade.

While many cells are known to contain stretch-sensitive ion channels, such as endothelial vascular epithelium,[17,46–49] the atomic structure of only a few is known, and molecular dynamics simulations prove somewhat inconclusive. Furthermore, only recently are efforts being made to identify the downstream effects of such mechanotransducive events in other cells and tissues.

The cellular response to force can vary from being essentially instantaneous to on the order of days and even years as with heart disease, for example.[50] Most rapid responses to force occur through transient changes in the transmembrane electric potentials because of ion flux through transmembrane channels[51] (Figure 2). The complete electrochemical impulse can be completed on the order of milliseconds.[19,20] These stretch-sensitive ion channels may be mechanically coupled to the cytoskeleton, or may be sensitive to membrane tensions (Figure 2).

It has also been demonstrated that changes in membrane fluidity during cell stretching can directly influence associations of transmembrane receptor molecules.[52–54] The time scale for these events is not known, but could be potentially immediate. Much research is surfacing to support that these events are possible due to force activated conformational

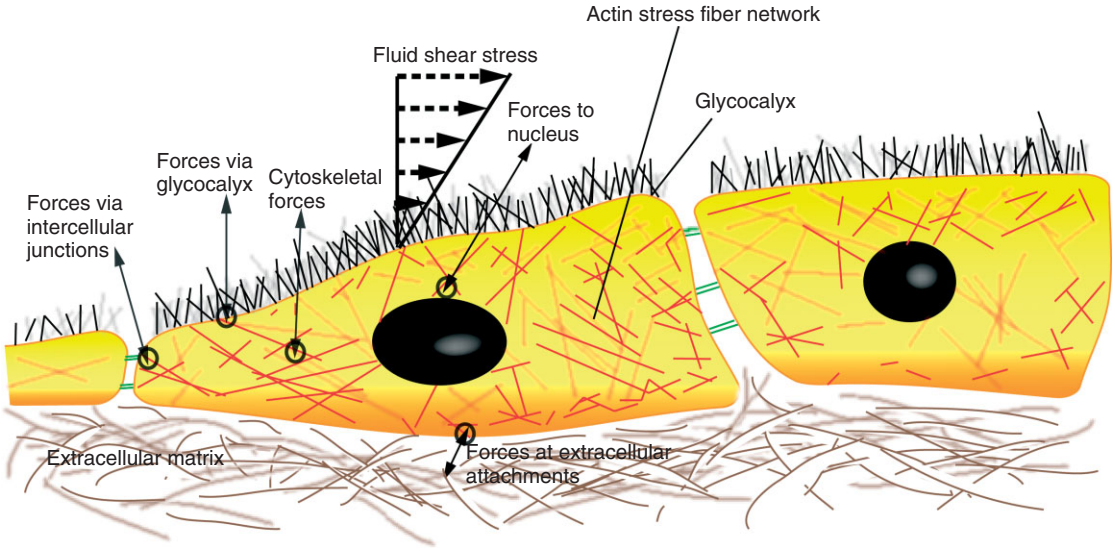

FIGURE 2 | A schematic illustration of some candidates that sense force and propagate the signaling cascade. While this illustration is not an exhaustive list of every candidate known to be sensitive to force, it describes some canonical mechanotransduction pathways, such as through the integrin–cytoskeleton linkage, and through stretch-sensitive ion channels.

changes of proteins, directly influencing their binding affinities. Force could alter the binding affinities for cytoskeletal binding proteins at focal adhesion complexes, for instance. Eventually, these conformational changes could potentially lead to activation of multiple signal transduction pathways and alter gene expression patterns.

A major misconenception of mechanosensation put to rest by Davies was that mechanosensation had to occur at the site of force application.[37] Davies illustrated that stresses, such as fluid shear stress, can be transmitted to remote parts of the cell through various intracellular structures.[37] To elaborate, the cytoskeleton is a highly interconnected web that connects cell–cell junctions, focal adhesions, the nuclear membrane, and cytoskeletal binding factors (Figure 2). Thus, any force at one site in the cell can be immediately propagated through the network and signal transduction can occur at any of these sites instantly. Researchers have even demonstrated that this can actually be many orders of magnitude faster than intraceullular biochemical cascades.[43]

SIGNALING THROUGH FOCAL ADHESIONS

Many biological researchers today appreciate that other proteins, in addition to transmembrane channels, undergo conformational changes when subjected to stress. Arguably the most widely known and studied mechanism of force sensation and transduction is through focal adhesion complexes. Focal adhesions bridge the extracellular matrix to the intracellular environment. Integrins are transmembrane heterodimeric transmembrane receptors that bind extracellular matrix molecules and link them to intraceullar cytoskeletal binding proteins like talin, vinculin, filamin, paxilin, and focal adhesion kinase (FAK), for example.[7,55–57] These cytoskeletal binding proteins bridge integrins to the F-actin cytoskeleton.

The integrin's cytoplasmic domain is associated with cytoskeletal binding proteins and can also be activated by force to associate with a host of second messengers to initiate signaling cascades. This signal cascade will not only recruit more cytoskeletal associations to increase mechanical bracing, but also increase the expression of focal adhesions and localization of more focal adhesions to the area. The response to force at a focal adhesion is logical, which increases its strength and the cell's ability to withstand deformation. However, there is a limit to this response which will be discussed in greater detail in subsequent sections.

The entire protein–protein interaction map is not known at focal complexes, but some examples are summarized in Figure 3. It is important to note that the nature and regulation of force-induced activation may differ drastically between these illustrated factors (Figure 3). One example illustrates how integrin receptors can activate FAK, either directly or by FAK's independently sensing force. A conformational change of FAK may alter its binding affinity for one of several

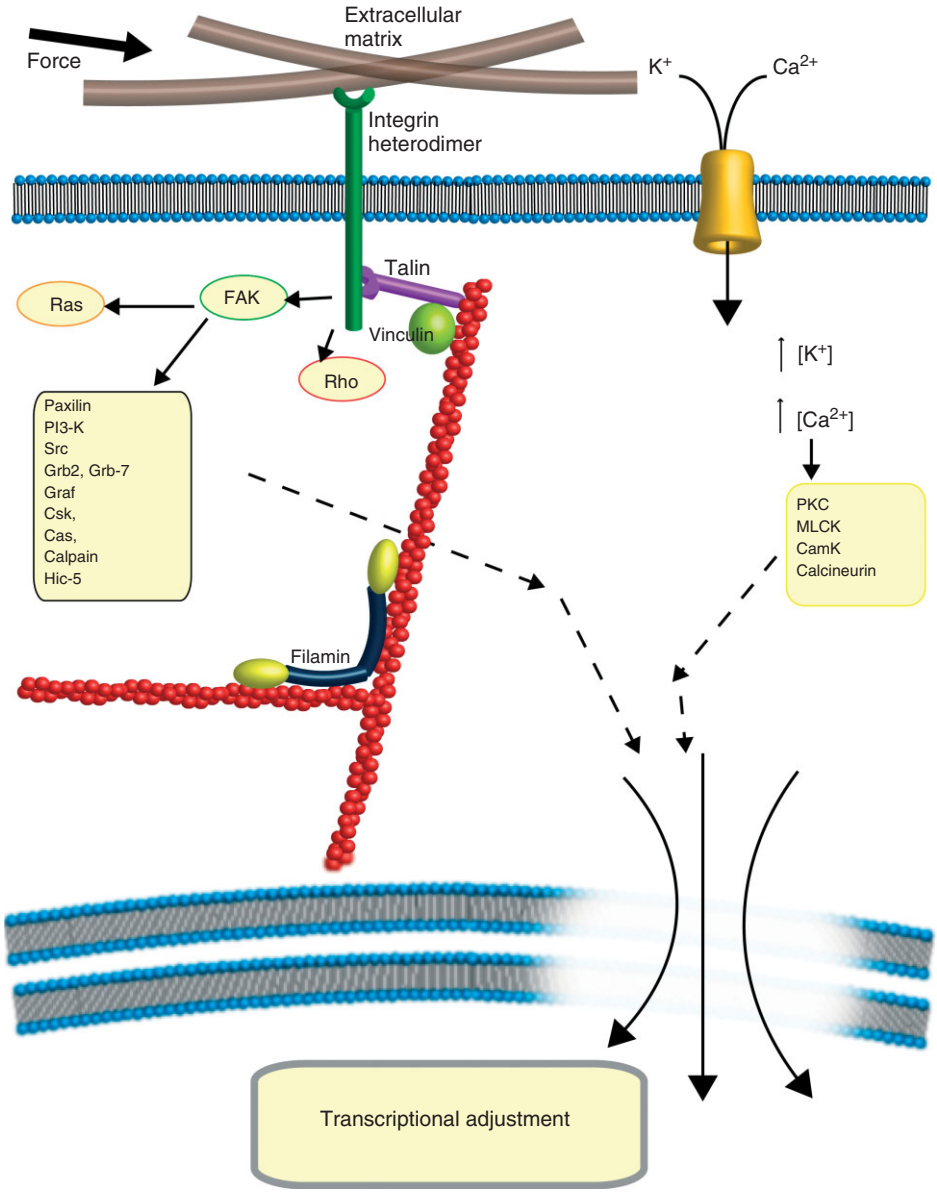

FIGURE 3 | Mechanotransduction can occur in many locations simultaneously within the cell. Forces can be transmitted from the exterior via the extracellular matrix or cell–cell junctions, or directly into structures like the glycocalyx. These transmitted forces can effect membrane fluidity and biochemistry, or propagate further through the cytoskeleton and even deform the nucleus. All of these events may occur in concert and their contribution need not be equivalent or even cooperative.

known binding partners. In addition, FAK activity may vary after the application of force, and this can in turn lead to the activation of other signaling molecules such as Rho, Rac, and Ras, to name a few.[58]

The experience of force at a focal adhesion not only leads to recruitment of cytoskeletal binding proteins, but also increases the number of recruited signal transduction molecules. A focal contact that has progressed to accumulate a number of molecular factors is termed a focal complex.

NUCLEAR SIGNALING

The nesprins are a family of proteins that link the cytoskeleton to the nucleus.[59–61] Nesprin 1 and 2 associate with actin filaments and SUN domain containing proteins of the nuclear membrane.[59–61] Nesprin 3 can also associate with SUN domain containing proteins, but links to the intermediate filament network.[59–61] The lamins are proteins that form the nuclear envelope, or stabilize intranuclear structure and associate with the SUN domain containing proteins and with chromatin associated factors.[59–61] Therefore, forces at the extracellular matrix can immediately propagate into the nucleus (Figure 3). This may initiate changes in transcription and subsequent changes in translation.

Additionally, forces propagated to the nucleus may physically alter the rate of nuclear cytoplasmic transport.[62] Thus, multiple means exist to sense forces

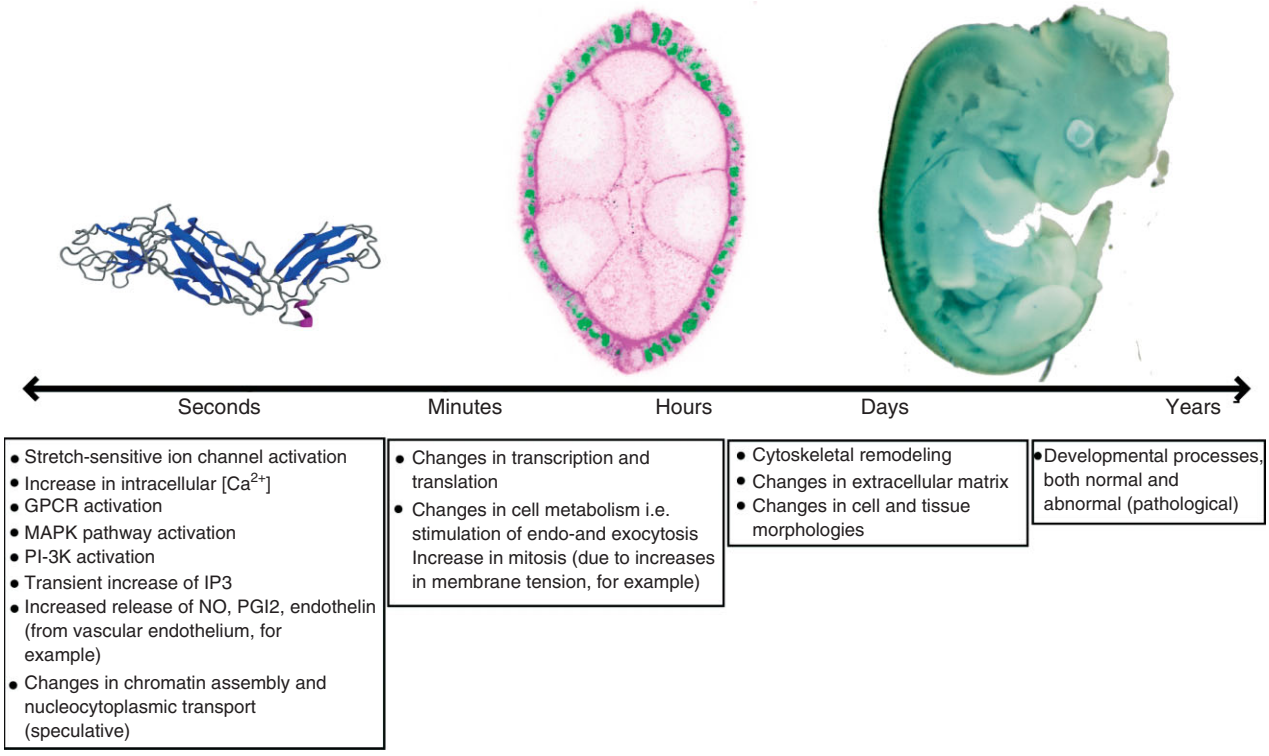

FIGURE 4 | The varying time and length scales of cellular responses are illustrated in this figure. In general, as time increases so do the macroscopic changes to the organism. The events that characterize short and quick responses, tend to be second messenger cascades or electrochemical. Other changes, such as gene transcription and cell/tissue morphological changes occur on the order of minutes to hours. Finally, if these events continue the final effect is large-scale changes in tissue organizations and morphological development of the organism. (Adapted with permission from Ref 65. Copyright 2003 BioMed Central).

at the nucleus; the topology of DNA can be directly affected, or the transport of transcription factors into and out of the nucleus may differ.

An increasing amount of research is demonstrating the grand role force has on cellular homeostasis and function. Cells often respond to force by modifying intracellular structure, migration, and modifying transcription and translation. More recently, researchers have also revealed another exciting front in mechanotransduction where forces are even thought to influence post-translation modifications. Indeed, interdisciplinary researchers employ biochemical knowledge of glycosylation to better understand how the turnover rate of the glycocalyx is influenced by shear stresses, for example.[63,64]

FORCE AND LENGTH SCALES OF MECHANOTRANSDUCTION

An interesting aspect of mechanotransduction that contrasts with other canonical cellular signal transduction pathways is the various responses that can be achieved with a seemingly uniform ligand/stimulus: force. This is surprising given that the nature of the cellular response to force can vary not only between differing cell types, but even within a uniform cell type. This is possible partly because cells and tissue experience forces of great range in magnitude and time scale or frequency (Figure 4).

Utilizing an *in situ* assay for the exposure of shielded or buried cysteine residues in proteins in cells, Johnson et al. were able to demonstrate that cytosolic proteins do unfold in their normal cellular environment.[66] Surprisingly, in both red blood cells, cells that regularly experience fluidic shear stress, and mesenchymal stem cells, there existed proteins that regularly unfolded.

While it is true for the most part that forces within tissues and cells are distributed and decrease with increasing distance from the site of application, cellular structure indicates means by which forces can be directly transmitted large distances and even concentrated. For example, cells are attached to the extracellular environment at discrete locations, focal contacts being one type. A uniformly applied stress to the top surface of a cell is inherently concentrated as

a consequence of there being only 1–10% of surface area contact with the substrate at the bottom surface. Thus, a stress can be magnified by many orders of magnitude.[39]

Mack et al. have demonstrated such stress focusing at focal contacts through cell force application with fibronectin coated magnetic beads.[39] In fibroblast cultures, the effect of focal adhesion stress focusing has also been reproduced.[67]

Atomic force microscopy and optical traps are experimental tools that can apply forces small enough to slowly extend proteins and probe interactions via force. In cases of extracellular adhesions, it has been shown that integrin–extracellular matrix component adhesions can be ruptured with forces on the order of 30–100 pN.[68] Proteins are viscoelastic and hence the strain rate markedly affects the unfolding pathway of the protein as well as the force required for unfolding.

Using molecular dynamics simulation and atomic force microscopy, mechanical linking proteins like titin in muscle, filamin, and α-actinin in F-actin cross linking, and talin in focal adhesion formation have been actively studied for their ability to sense force inputs.[55–57,69,70] The forces for many of the conformational changes exhibited by these proteins fall within the range of 10–100 pN and occur on the order of picoseconds to microseconds. The sensation of force by these proteins must be greater than the thermal energy available to the system (kT) which is approximately 4 pN·nm.

Researchers have quantified the force that cells actively contract on their substrate, which is measured to approximately 5.5 nN/μm^2.[71,72] This measurement has made possible the extrapolation of the force experienced by a single integrin molecule to be on the order of several piconewtons. Mack et al. have shown that forces applied via magnetic beads to adherent vascular endothelial cells can elicit a response if on the order of 1 nN, corresponding to the threshold of 1 Pa that Davies et al. proposed to approximate the threshold for stimulation by hemodynamic shear stress.[37,39]

The body experiences forces on time scales that are many orders of magnitude greater than what cells and molecules require to respond to mechanical stimuli. This can be analogous to having cells, which typically have transmembrane electrical potentials on the order of hundreds of millivolts, to experiencing external electrical fields on the order of volts to kilovolts on a daily basis.

Just as chemical physiological processes exist in the organism to preserve homeostasis, processes must exist to preserve homeostasis in light of the many mechanical stimuli that an organism experiences on a daily basis. These effects must be remarkably robust, yet exquisitely sensitive to account for the staggering ranges in force, length, and time scales experienced.

MECHANOTRANSDUCTION IN EPITHELIAL TISSUES

In 1824, Ètienne Serres put forth the embryological parallelism 'ontogeny recapitulates phylogeny'.[73] Consistent with the Meckel–Serres Law, epithelial tissue is considered to be one of the first tissues to evolve and is the first tissue formed during development. Furthermore, epithelial tissue is believed to be one of the most basic tissue types, and therefore can serve as an excellent model for research in 'developmental biomechanics'.

Epithelium occurs on all inner and outer surfaces of organs. During development, epithelial cells form confluent monolayers and these epithelial tissue sheets twist and contort to form various structures to give rise or house developing organs and bodily compartments.[74]

Epithelial cells have an apical face and a basal face (Figure 5). The apical face functions in absorption and secretion of matter from or into the luminal space formed by the epithelial tissue. The basal face of the epithelium is the site attachment to the ECM, a complex network of post-translationally modified protein filaments, such as collagens, laminins, fibrins, fibrilarins, and many more[6,9] (Figure 5). ECM filaments are highly charged because of extensive post-translational modifications such as glycosylation and sulfation, and are given the name glycosaminoglycans. The extensive charge of the ECM underlies its water retaining properties and hence directly influences the viscoelasticity. In aging the number of glycosaminoglycans or overall charge of the ECM tends to decrease reducing the tissue viscoelasticity.

Epithelial tissue sheets are organized by their expression of adhesional proteins on their membrane surface, primarily their lateral membrane surfaces (Figure 5). Many adhesional protein complexes exist within epithelial cells and they can have varying degrees of adhesion strength. Cellular adhesion is highly regulated and is organized in specific orientations and geometries giving rise to many integral functional properties of the epithelium.

One adhesional structure that is integral to tissue architecture and highly studied is the zonula adherens, or adherens junctions. The proteins responsible for cell–cell adherens junctions are the calcium ion dependent adhesion molecules, the cadherins. Many different isoforms or homologs exist, and exhibit differing pairing affinities. When two cells adhere

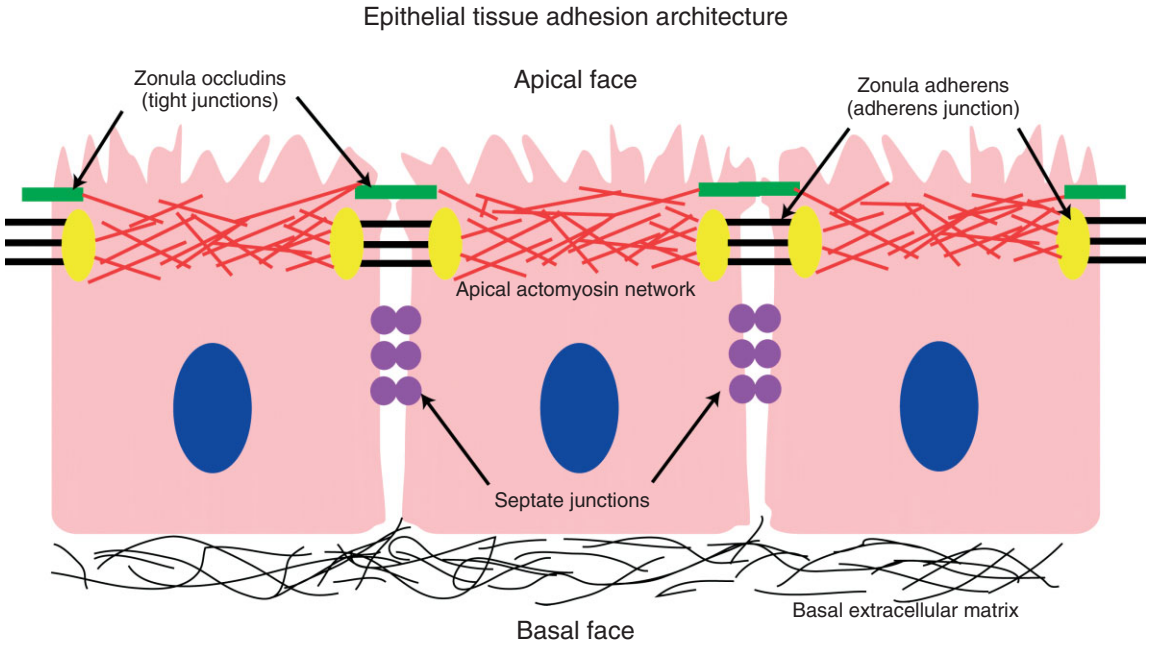

FIGURE 5 | A generalized epithelial tissue architecture depicting some characteristic epithelial adhesion structures and geometry. The epithelial tissue is polarized with an apical face and a basal face. The apical face functions mainly to import and export. To increase surface area, the membrane is highly folded forming structures called villi and within the villi are even smaller microvilli. At the basal domain is the extracellular matrix that forms the tissue scaffold. Three major cell–cell adhesion structures exist each with different accompanying physiological functions, the tight junctions, adherens junctions, and the septate junctions. All of these junctions also serve as intracellular anchor points for the cytoskeleton, commonly actin, microtubules, and intermediate filaments.

to one another via cadherins, the adhesive complex formed is termed a desmosome, or macula adherentes, in Latin meaning 'adhering spot'.

Interestingly, the desmosomes formed between two cells need not be between identical cadherin isoforms. Cadherins have been shown to form not only homodimers between cells, but also heterodimers between isoforms of markedly differing affinities.[75,76] Consequentially, epithelial cells can prefer to adhere more tightly to neighbors expressing isoforms that maximize adhesional strength.[75,77] This hypothesis was proposed originally by Steinberg given this heterodimerization phenomenon, and was named the differential adhesion hypothesis (DAH).[78,79]

The DAH proposes that epithelial tissue morphogenesis can be driven by the segregation of epithelial cells due to expression of differing cadherins.[78,80,81] In other words, two differing cell types can form immiscible, separate clusters solely based upon their differing adhesion affinities.[76] It also proposes that to interchelate cell types, similar adhesion affinities must exist.[75,78] The DAH is often described similar to the theory of chemical solubility, in which similar interactions, or interaction affinity between particles should promote solubility. However, recent work has revealed that the affinities of cadherins expressed by cells do not correlate with the predictions of cell lineage segregation.[76,82]

Nevertheless, the DAH has been demonstrated to be consistent with a number of morphogenetic processes,[80,81] and this inconsistency has spurred a number of researchers to propose that active processes may be at play.[75,82] One of the major active processes that is proposed to be at play is mechanotransduction, and many investigations support that the cadherins may respond to force, namely β-catenin.[82] When cyclical stress is applied to cells expressing β-catenin, the stiffness of the cells increases, due in part to the increased recruitment of filamentous actin to cadherins.[82] Although these experimental observations support that β-catenin can function as a mechanosensor, it does not directly reveal why adhesion affinities do not correlate to cell–cell segregation predictions. However, these works do demonstrate the dynamic nature between the cytoskeleton and intercellular interactions.

As the cytoskeleton is responsible for cell shape, adhesion therefore also affects the geometry of the cell by interacting with and modifying the cytoskeleton.[83] Interestingly, this effect is reciprocal, as the cytoskeleton can exert stresses on adhesion structures, and promote their disassembly, for example.[81]

CELL AND TISSUE MORPHOGENESIS RELIES ON MECHANICAL CUES

During development of multicellular organisms, epithelial cell sheets coordinate to achieve a myriad of specific and distinctive shape changes. Invagination, evagination, folding, intercalation (convergent extension), cell flattening (epiboly), ingression, egression, and branching are widespread examples of epithelial morphogenesis in the animal kingdom.[84–86] Remarkably, the epithelial cells within the tissue must precisely organize adhesion, actin–myosin contractility, apical–basal and planar cell polarity during morphogenesis.

During morphogenesis, each cell must process and physically respond to patterning and polarity information in order to effectively take part in tissue growth. Cell behaviors during morphogenesis include processes such as cell growth, migration, death, division, and shape changes. Biological tissues have two mechanical properties that directly contradict each other. They exhibit a stable structure, which is needed to resist stresses, but they also demonstrate a dynamic and plastic behavior, allowing for remodeling.[81] The balance of these two opposing mechanisms can be thought of as leading to tissue homeostasis.[81] The balance between intercellular adhesion, an outwardly directed force, and intracellular contractility, an inwardly directed force, can bring about tissue homeostasis. Cells can tilt the balance of forces to create polarized epithelial layers and control cell sorting.[81]

The orchestration of such mechanisms has been mainly studied through the identification of signaling pathways that control cell behavior both in time and space. However, it is an open area of investigation as to how this information is processed to control cell shape and dynamics. A range of developmental phenomena can be explained by the regulation of cell surface tension through adhesion and cortical actin networks.[81]

Although the interplay between adhesion and contractility can explain or support a number of developmental phenomena, it is unknown how this can be exploited or exactly how this leads to the observed morphogenesis, i.e., by identifying molecular candidates and their spatiotemporal expression. In addition, describing certain developmental or pathological mechanisms solely by adhesion and contractility variations, such as in cellular metastasis or mesenchymal cell migrations, may be too oversimplifying to be useful. Morphogenesis ultimately relies upon the mechanical environment for cues.

STEM CELL MORPHOGENESIS

If development and morphogenesis is exquisitely sensitive to mechanical cues, it is not surprising that these cues are equally influential in stem cell differentiation. It has been repeatedly demonstrated that stem cell differentiation can be reliably controlled solely by mechanical cues.[5,87–90] Although *in vivo* stem cell differentiation likely involves more than just mechanical cues, i.e., biochemical cues as well, this mechanically driven differentiation is robust and can be reproduced reliably.

In general, research in this field is supporting that stem cells differentiate to whatever tissue stiffness most resembles that of their substrate.[6] For example, nervous tissue is comparatively soft to other human tissues, and neurons therefore form from stem cells when cultured on soft substrates.[87,88,90,91] Myocytes and osteoblasts form on substrates that are increasingly stiff.[6] Moreover, these phenomena occur in multiple stem cell lineages, and even in circulating stem cells.[90]

Even cells that are not stem cells tend to mimic the stiffness of their substrate, such as fibroblasts.[92] Fibroblasts within a sensible range, will contract on their substrate such that their average cellular stiffness will reflect that of the substrate.[92] Fibroblasts as a mature cell line may be unique in this capacity as they generally serve a repair or wound healing role in the body. Therefore, it is conceivable that to form repair tissues, it would be ideal to most closely resemble what was previously lost or damaged.

TUMOROGENESIS

Cells can misinterpret mechanical cues and become malignant. In some cases, like breast cancer, for instance, the stiffness of the substrate directly causes female mouse mammary epithelium to become malignant.[93,94] Even more astonishing is that the malignant cells can be transferred to soft substrates, resembling normal physiological stiffness, and the cells will return to normal. It is hypothesized that similar mechanisms exist underlying the progression of one of the most deleterious forms of cancer, glioblastoma multiforme.[95]

It is possible that mechanical cues can potentiate an existing or new mutation allowing pathogenecity, but this is unknown. A defining characteristic of nearly all tumors is the increased expression of matrix metalloproteases.[96,97] Matrix metalloproteases are widely expressed in metastatic cancers, as a means to potentially create a greater void for tumor proliferation, or possibly to modify their mechanical

environment for other means. This is an active area of research with much still remaining to be investigated.

CONCLUSIONS: AN INTEGRATION OF MECHANOTRANSDUCTION FROM MOLECULAR, CELLULAR, TISSUE AND ORGAN/ORGANISMAL LEVELS

Mechanotransduction is a relatively newly discovered phenomenon describing the translation of forces into a biochemical language the cell can understand. Cells and tissues are extremely sensitive to changes in their mechanical environment and respond in ways that are still explicitly unclear.

Cells employ more than just chemical cues to regulate their physiology, and mechanics plays an integral role in regulating cell movements, morphologies, and even metabolism. During development, a number of complex cell shape changes and population reorganizations occur to result in the complex tissue geometries of the organism. These involve coordinated movements of cells and massive matrix remodeling to form these tissues. Mechanotransduction undoubtedly plays a role during these processes.

Just as in other signal transduction pathways, feedback loops exist to promote homeostasis. A mechanical response to the environment, such as decreasing cellular contractility or cytoskeletal integrity, or increasing activity matrix degradation or deposition, can significantly adjust the mechanical environment. This can in turn, lead to a reciprocal response within that same cell, or lead to a change in nearby cell populations.[9]

The implications of this are astounding, and can, potentially, lead to new theories in areas of tissue engineering, regenerative medicine, and development. For example, although stem cells can be driven via mechanical changes to differentiate to target cell types or some tissues, no methods exist to create complete biological organs.[98] Currently the major hurdle is culturing large tissues in three dimensions.[98]

In addition to this major problem, it is also necessary to interchelate multiple tissue types and cell types into a defined three-dimensional topology for proper organ function. For example, vascular tissue must be present in all target organs.[99] The process by which these tissues organize themselves and differentiate into particular shapes and topologies are unknown. Cell environments are dynamic during *in vivo* development and morphogenesis, and nor can current approaches capture this. A cell's environment is constantly being reshaped and therefore tissue engineering *in vitro* must also be dynamic.[89]

It is often neglected that many of the forces and mechanical cues of a developing organ or tissue may come from tissues not necessarily from the same lineage. In fact, it is entirely possible that mechanotransduction can occur between tissues, or intertissue mechanotransduction. A fundamental understanding of the *in vivo* micromechanics of processes involved in morphogenesis is essential to employ these processes *in vitro* to differentiate stem cells to a target tissue.

Understanding how a mechanical, dynamic feedback between interacting tissues can drive concerted development during reproductive growth is integral for future prospects in regenerative medicine.[89] For example, the mechanical identification that neurons regulate axonal growth by tension to connect to other neurons, has allowed for the creation of devices that can in a user-defined manner, guide neuron axonal growth both *in vitro* and *in vivo*.[11,100] With a single observation in the mechanics of development of neural connective systems, these devices were fabricated and now make possible the repair of nerve connections in the human nervous system *in situ*.[11]

It may be advantageous, then, to take a top–down approach rather than a bottom up in regards to tissue and organogenesis. The assumption that fully developed organs are modular may not be valid during development. Organs may require all accessory tissues with which they develop, despite these tissues not being intimately involved in organ function, nor derived from the same germ layer. Research may benefit to test if developing tissues can be shaped in a controlled manner.

In addition, the magnitude of developmental factors omitted during classical *in vitro* regenerative medicine applications is largely confounding in our efforts to understand differentiation. It may serve research better, to instead allow development to proceed, but aim to precisely guide cell movements by varying select parameters. This method, *in situ* directed tissue engineering, would benefit in allowing the researcher to make direct cause and effect relationships, thus enhancing the wealth of knowledge in directed growth. *In situ* directed tissue engineering efforts could test if ontogeny can be recapitulated and modified *in vitro*.

Many pathologies and abnormalities can surface when mechanotransduction malfunctions, including but not limited to tumorogenesis. For an excellent review on pathological conditions resulting from improper mechanotransduction, see Jaalouk and Lammerding.[34]

Physical changes described by mechanics are the ultimate means tissues communicate, and changes in these mechanics describe changes in spatial and

temporal organizations of tissues, supporting the biological function. Organogenesis thus requires a greater understanding of the relationships between the biochemistry and their net physical effects.

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REFERENCES

1. Raya A, et al. Notch activity acts as a sensor for extracellular calcium during vertebrate left-right determination. *Nature* 2004, 427:121–128.
2. Goodwin M, Yap AS. Classical cadherin adhesion molecules: coordinating cell adhesion, signaling and the cytoskeleton. *J Mol Histol* 2004, 35:839–44.
3. Iqbal J, Zaidi M. Molecular regulation of mechanotransduction. *Biochem Biophys Res Commun* 2005, 328:751–755.
4. Ingber DE. Cellular mechanotransduction: putting all the pieces together again. *FASEB J* 2006, 20:811–827.
5. Ingber DE. The mechanochemical basis of cell and tissue regulation. *Mech Chem Biosyst* 2004, 1:53–68.
6. Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. *Science* 2005, 310:1139–1143.
7 Mofrad M, Kamm R. *Cellular Mechanotransduction: Diverse Perspectives from Molecules to Tissuesy*. New York: Cambridge University Press; 2009, 375.
8. Lopez JI, Mouw JK, Weaver VM. Biomechanical regulation of cell orientation and fate. *Oncogene* 2008, 27:6981–6993.
9. Butcher DT, Alliston T, Weaver VM. A tense situation: forcing tumour progression. *Nat Rev Cancer* 2009, 9:108–122.
10. Robertson MJ, Gip P, Schaffer DV. Neural stem cell engineering: directed differentiation of adult and embryonic stem cells into neurons. *Front Biosci* 2008, 13:21–50.
11. Phillips JB, et al. Neural tissue engineering: a self-organizing collagen guidance conduit. *Tissue Eng* 2005, 11:1611–1617.
12. Marshall WF, Kintner C. Cilia orientation and the fluid mechanics of development. *Curr Opin Cell Biol* 2008, 20:48–52.
13. Taber LA. Biophysical mechanisms of cardiac looping. *Int J Dev Biol* 2006, 50:323–332.
14. Voronov DA, et al. The role of mechanical forces in dextral rotation during cardiac looping in the chick embryo. *Dev Biol* 2004, 272:339–350.
15. Dewey CF Jr, et al. The dynamic response of vascular endothelial cells to fluid shear stress. *J Biomech Eng* 1981, 103:177–185.
16. Hahn C, Schwartz MA. Mechanotransduction in vascular physiology and atherogenesis. *Nat Rev Mol Cell Biol* 2009, 10:53–62.
17. Barakat AI. Responsiveness of vascular endothelium to shear stress: potential role of ion channels and cellular cytoskeleton (review). *Int J Mol Med* 1999, 4:323–332.
18. Barbee KA, et al. Subcellular distribution of shear stress at the surface of flow-aligned and nonaligned endothelial monolayers. *Am J Physiol* 1995, 268:H1765–H1772.
19. Lehoux S, Tedgui A. All strain, no gain: stretch keeps proliferation at bay via the NF-kappaB response gene iex-1. *Circ Res* 2003, 93:1139–1141.
20. Lehoux S, Tedgui A. Cellular mechanics and gene expression in blood vessels. *J Biomech* 2003, 36:631–643.
21. Kassab GS. "Bert" Fung: the father of modern biomechanics. *Mech Chem Biosyst* 2004, 1:5–22.
22. Kumar S, Weaver VM. Mechanics, malignancy, and metastasis: the force journey of a tumor cell. *Cancer Metastasis Rev* 2009, 28:113–127.
23. Discher D, et al. Biomechanics: cell research and applications for the next decade. *Ann Biomed Eng* 2009, 37:847–859.
24. Fernandez-Gonzalez R, Zallen JA. Epithelial organization: may the force be with you. *Curr Biol* 2008, 18:R163–R165.
25. Huveneers S, Danen EH. Adhesion signaling—crosstalk between integrins, Src and Rho. *J Cell Sci* 2009, 122:1059–1069.
26. Lindberg U, et al. The microfilament system and malignancy. *Semin Cancer Biol* 2008, 18:2–11.
27. Jimènez-Baranda S, et al. Filamin-A regulates actin-dependent clustering of HIV receptors. *Nat Cell Biol* 2007, 9:838–846.
28. Bateman J, et al. The receptor tyrosine phosphatase Dlar and integrins organize actin filaments in the

Drosophila follicular epithelium. *Curr Biol* 2001, 11:1317–1327.

29. Deng WM, et al. Dystroglycan is required for polarizing the epithelial cells and the oocyte in Drosophila. *Development* 2003, 130:173–184.
30. Frydman HM, Spradling AC. The receptor-like tyrosine phosphatase lar is required for epithelial planar polarity and for axis determination within drosophila ovarian follicles. *Development* 2001, 128:3209–3220.
31. Gutzeit HO. The microfilament pattern in the somatic follicle cells of mid-vitellogenic ovarian follicles of Drosophila. *Euro J Cell Bio* 1990, 53:349–356.
32. Gimbrone MA, et al. Endothelial dysfunction, hemodynamic forces, and atherogenesis. *Ann N Y Acad Sci* 2000, 902:230–239; discussion 239–40.
33. Haga JH, Li YS, Chien S. Molecular basis of the effects of mechanical stretch on vascular smooth muscle cells. *J Biomech* 2007, 40:947–960.
34. Jaalouk DE, Lammerding J. Mechanotransduction gone awry. *Nat Rev Mol Cell Biol* 2009, 10:63–73.
35. Lammerding J, Kamm RD, Lee RT. Mechanotransduction in cardiac myocytes. *Ann N Y Acad Sci* 2004, 1015:53–70.
36. Li YS, Haga JH, Chien S. Molecular basis of the effects of shear stress on vascular endothelial cells. *J Biomech* 2005, 38:1949–1971.
37. Davies PF. Flow-mediated endothelial mechanotransduction. *Physiol Rev* 1995, 75:519–560.
38. Dai G, et al. Distinct endothelial phenotypes evoked by arterial waveforms derived from atherosclerosis-susceptible and -resistant regions of human vasculature. *Proc Natl Acad Sci U S A* 2004, 101:14871–14876.
39. Mack PJ, et al. Force-induced focal adhesion translocation: effects of force amplitude and frequency. *Am J Physiol Cell Physiol* 2004, 287:C954–C962.
40. Boussel L, et al. Aneurysm growth occurs at region of low wall shear stress: patient-specific correlation of hemodynamics and growth in a longitudinal study. *Stroke* 2008, 39:2997–3002.
41. Mofrad MRK, et al. Characterization of the atherosclerotic carotid bifurcation using MRI, finite element modeling, and histology. *Ann Biomed Eng* 2004, 32:932–946.
42. Tzima E, et al. Activation of Rac1 by shear stress in endothelial cells mediates both cytoskeletal reorganization and effects on gene expression. *EMBO J* 2002, 21:6791–6800.
43. Wang N, Tytell JD, Ingber DE. Mechanotransduction at a distance: mechanically coupling the extracellular matrix with the nucleus. *Nat Rev Mol Cell Biol* 2009, 10:75–82.
44. Santos A, Bakker AD, Klein-Nulend J. The role of osteocytes in bone mechanotransduction. *Osteoporos Int* 2009, 20:1027–1031.
45. Holt JR, Corey DP. Two mechanisms for transducer adaptation in vertebrate hair cells. *Proc Natl Acad Sci U S A* 2000, 97:11730–11735.
46. Hamill OP. Twenty odd years of stretch-sensitive channels. *Pflugers Arch* 2006, 453:333–351.
47. Ninio DM, Saint DA. The role of stretch-activated channels in atrial fibrillation and the impact of intracellular acidosis. *Prog Biophys Mol Biol* 2008, 97:401–416.
48. Suchyna TM, Sachs F. Mechanosensitive channel properties and membrane mechanics in mouse dystrophic myotubes. *J Physiol* 2007, 581:369–387.
49. Zagorodnyuk VP, et al. Mechanotransduction and chemosensitivity of two major classes of bladder afferents with endings in the vicinity to the urothelium. *J Physiol* 2009, 587:3523–3538.
50. Weinberg EJ, Schoen FJ, Mofrad MR. A computational model of aging and calcification in the aortic heart valve. *PLoS One* 2009, 4:e5960.
51. Ingber DE. Tensegrity-based mechanosensing from macro to micro. *Prog Biophys Mol Biol* 2008, 97:163–179.
52. Li S, et al. The role of the dynamics of focal adhesion kinase in the mechanotaxis of endothelial cells. *Proc Natl Acad Sci U S A* 2002, 99:3546–3551.
53. Sawada Y, Sheetz MP. Force transduction by Triton cytoskeletons. *J Cell Biol* 2002, 156:609–615.
54. Zhang YL, Frangos JA, Chachisvilis M. Mechanical stimulus alters conformation of type 1 parathyroid hormone receptor in bone cells. *Am J Physiol Cell Physiol* 2009, 296:C1391–C1399.
55. Golji J, Collins R, Mofrad MR. Molecular mechanics of the alpha-actinin rod domain: bending, torsional, and extensional behavior. *PLoS Comput Biol* 2009, 5:e1000389.
56. Kolahi KS, Mofrad MR. Molecular mechanics of filamin's rod domain. *Biophys J* 2008, 94:1075–1083.
57. Lee SE, et al. Molecular dynamics study of talin-vinculin binding. *Biophys J* 2008, 95:2027–2036.
58. Katsumi A, et al. Integrins in mechanotransduction. *J Biol Chem* 2004, 279:12001–12004.
59. Crisp M, et al. Coupling of the nucleus and cytoplasm: role of the LINC complex. *J Cell Biol* 2006, 172:41–53.
60. Haque F, et al. SUN1 interacts with nuclear lamin A and cytoplasmic nesprins to provide a physical connection between the nuclear lamina and the cytoskeleton. *Mol Cell Biol* 2006, 26:3738–3751.
61. Warren DT, et al. Nesprins: intracellular scaffolds that maintain cell architecture and coordinate cell function? *Expert Rev Mol Med* 2005, 7:1–15.

62. Wolf C, Mofrad MR. On the octagonal structure of the nuclear pore complex: insights from coarse-grained models. *Biophys J* 2008, 95:2073–2085.
63. Lopez-Quintero SV, et al. The endothelial glycocalyx mediates shear-induced changes in hydraulic conductivity. *Am J Physiol Heart Circ Physiol* 2009, 296:H1451–H1456.
64. Tarbell JM, Ebong EE. The endothelial glycocalyx: a mechano-sensor and -transducer. *Sci Signal* 2008, 1:pt8.
65. Yan L, et al. Knockout of ERK5 causes multiple defects in placental and embryonic development. *BMC Dev Biol* 2003, 3:11.
66. Johnson CP, et al. Forced unfolding of proteins within cells. *Science* 2007, 317:663–666.
67. Hu S, et al. Intracellular stress tomography reveals stress focusing and structural anisotropy in cytoskeleton of living cells. *Am J Physiol Cell Physiol* 2003, 285:C1082–C1090.
68. Lehenkari PP, Horton MA. Single integrin molecule adhesion forces in intact cells measured by atomic force microscopy. *Biochem Biophy Res Commun* 1999, 259:645–650.
69. Oberhauser AF, et al. Stepwise unfolding of titin under force-clamp atomic force microscopy. *Proc Natl Acad Sci U S A* 2001, 98:468–472.
70. Schwaiger I, et al. A mechanical unfolding intermediate in an actin-crosslinking protein. *Nat Struct Mol Biol* 2004, 11:81–85.
71. Balaban NQ, et al. Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. *Nat Cell Biol* 2001, 3:466–472.
72. Geiger B, et al. Transmembrane crosstalk between the extracellular matrix–cytoskeleton crosstalk. *Nat Rev Mol Cell Biol* 2001, 2:793–805.
73. Montgomery W. The politics of evolution. Morphology, medicine, and reform in radical London. Adrian Desmond. University of Chicago Press, Chicago, 1990. x, 503 pp., illus. $34.95. Science and its conceptual foundations. *Science* 1990, 248:883–884.
74. Marieb EN, Hoehn K. *Anatomy and Physiologyy*. Benjamin Cummings; 2007, 1048.
75. Leckband D. Beyond structure: mechanism and dynamics of intercellular adhesion. *Biochem Soc Trans* 2008, 36:213–220.
76. Shi Q, Chien YH, Leckband D. Biophysical properties of cadherin bonds do not predict cell sorting. *J Biol Chem* 2008, 283:28454–28463.
77. Lecuit T. Adhesion remodeling underlying tissue morphogenesis. *Trends Cell Biol* 2005, 15:34–42.
78. Foty RA, Steinberg MS. The differential adhesion hypothesis: a direct evaluation. *Dev Biol* 2005, 278:255–263.
79. Steinberg MS, Poole TJ. Strategies for specifying form and pattern: adhesion-guided multicellular assembly. *Philos Trans R Soc Lond B Biol Sci* 1981, 295:451–460.
80. Lecuit T, Le Goff L. Orchestrating size and shape during morphogenesis. *Nature* 2007, 450:189–192.
81. Lecuit T, Lenne PF. Cell surface mechanics and the control of cell shape, tissue patterns and morphogenesis. *Nat Rev Mol Cell Biol* 2007, 8:633–644.
82. Chien YH, et al. Two stage cadherin kinetics require multiple extracellular domains but not the cytoplasmic region. *J Biol Chem* 2008, 283:1848–1856.
83. Ingber DE. Mechanobiology and diseases of mechanotransduction. *Ann Med* 2003, 35:564–577.
84. Fristrom D. The cellular basis of epithelial morphogenesis. A review. *Tissue Cell* 1988, 20:645–690.
85. Pilot F, Lecuit T. Compartmentalized morphogenesis in epithelia: from cell to tissue shape. *Dev Dyn* 2005, 232:685–694.
86. Quintin S, Gally C, Labouesse M. Epithelial morphogenesis in embryos: asymmetries, motors and brakes. *Trends Genet* 2008, 24:221–230.
87. Engler AJ, et al. Matrix elasticity directs stem cell lineage specification. *Cell* 2006, 126:677–689.
88. Engler AJ, et al. Extracellular matrix elasticity directs stem cell differentiation. *J Musculoskelet Neuronal Interact* 2007, 7:335.
89. Ghosh K, Ingber DE. Micromechanical control of cell and tissue development: implications for tissue engineering. *Adv Drug Deliv Rev* 2007, 59:1306–1318.
90. Zajac AL, Discher DE. Cell differentiation through tissue elasticity-coupled, myosin-driven remodeling. *Curr Opin Cell Biol* 2008, 20:609–615.
91. Thakar RG, et al. Contractility-dependent modulation of cell proliferation and adhesion by microscale topographical cues. *Small* 2008, 4:1416–1424.
92. Solon J, et al. Fibroblast adaptation and stiffness matching to soft elastic substrates. *Biophys J* 2007, 93:4453–4461.
93. Novaro V, Roskelley CD, Bissell MJ. Collagen-IV and laminin-1 regulate estrogen receptor alpha expression and function in mouse mammary epithelial cells. *J Cell Sci* 2003, 116:2975–2986.
94. Rønnov-Jessen L, Bissell MJ. Breast cancer by proxy: can the microenvironment be both the cause and consequence? *Trends Mol Med* 2009, 15:5–13.
95. Ulrich TA, de Juan Pardo EM, Kumar S. The mechanical rigidity of the extracellular matrix regulates the structure, motility, and proliferation of glioma cells. *Cancer Res* 2009, 69:4167–4174.
96 Tallant C, Marrero A, Gomis-Ruth FX. Matrix metalloproteinases: fold and function of their catalytic domains. *Biochim Biophys Acta* 2009, 15.

97. Wolf K, Friedl P. Mapping proteolytic cancer cell-extracellular matrix interfaces. *Clin Exp Metastasis* 2009, 26:289–298.

98. Tsang VL, Bhatia SN. Fabrication of three-dimensional tissues. *Adv Biochem Eng Biotechnol* 2007, 103:189–205.

99. Borenstein JT, et al. Microfabrication of three-dimensional engineered scaffolds. *Tissue Eng* 2007, 13:1837–1844.

100. Heidemann SR, Buxbaum RE. Tension as a regulator and integrator of axonal growth. *Cell Motil Cytoskeleton* 1990, 17:6–10.