FORUM EDITORIAL

Mechanotransduction: Forces, Sensors, and Redox Signaling

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Abstract

Mechanotransduction describes the molecular mechanisms by which cells respond to changes in their physical environment by translating mechanical stimuli into biochemical signals. It is now clear that reactive oxygen species (ROS) and redox signaling play a crucial role in mechanotransduction analogous to their role in chemotransduction. This Forum has particular emphasis on ROS generation with altered mechanical stress, the upstream signal transduction pathways that initiate ROS production, and the downstream effectors that lead to physiological responses. There is particular emphasis on the role of ion channels in the initial response and the role of NADPH oxidases as the major source of ROS. The latter enzyme serves as the fulcrum of the mechanotransduction cascade. Although it seems likely that all cells are mechanosensitive to some degree, we have highlighted the responses of unicellular organisms (bacteria), bone cells, and particularly cells of the vasculature (endothelial cells and vascular smooth muscle cells). These cell types have been useful for studying the responses to altered osmotic pressure, hemodynamic pressure, shear stress, and compressive forces while exploring the link between signal transduction and physiological/pathophysiological responses. *Antioxid. Redox Signal.* 20, 868–871.

MECHANOTRANSDUCTION IS BASED on the premise that cells respond to their environment by translating mechanical stimuli into biochemical signals. Indeed, mechanical forces arising from environmental factors, such as osmotic pressure, compression, tension, and fluid shear stress, are major regulators of cellular function. These forces are important to mediate cell development and to maintain homeostasis in blood vessels, bone, skeletal muscle, and other tissues. Several elements of the mechanotransduction cascade are redox sensitive, analogous to the responses to chemical signals (chemotransduction). These redox-sensitive signaling pathways enable cells to respond to their physical surroundings, providing feedback between cellular elements and the physical environment. The goals of this Forum are to describe these responses to various forms of mechanical stress, to promote understanding of common pathways for sensing and transducing the signals from the physical environment, and to emphasize the important role of redox systems in the signal transduction process. The signal transduction pathways that are discussed in the Forum articles and their relationship to reactive oxygen species (ROS) are shown schematically in Figure 1.

The article by Browning *et al.*, and the only original research presentation in this Forum, introduces the concept of

shear associated with blood flow as an important physical force in vascular biology. Since the 19th century, it has been accepted that rheological (physical/mechanical) principles are crucial for vascular homeostasis; indeed, the triad of blood–blood vessel–blood flow presented by the eminent German pathologist Rudolph Virchow formed the basis for early investigations of organ perfusion-associated pathologies. More than a century later, it has become clear that endothelial cells lining vessels sense the shear stress associated with blood flow and the resultant mechanosignaling regulates vascular diameter and vessel tone.

Shear stress in the vascular endothelium derives from the blood flow that can be laminar, oscillatory, or disturbed (turbulent). The majority of *in vitro* studies have evaluated endothelial cells cultured *in vitro* under static conditions and then exposed to laminar flow. As normal endothelium in intact organs is expected to be in a flow-adapted state (adapted to prolonged periods of constant shear), these studies using statically cultured cells are inadequate to fully understand physiological mechanosignaling as would occur *in vivo*. In a departure from the onset of flow paradigm, the current contribution of Browning *et al.* and previous reports from this group have studied stop of flow in cells that have undergone prior flow adaptation. This work has shown that abrupt loss of

FIG. 1. Schematic representation indicating the signal transduction pathway in response to altered mechanical forces, the central role for the generation of reactive oxygen species (ROS), and the downstream physiological responses.

flow results in essentially immediate inactivation of the endothelial K_{ATP} channel with consequent cell membrane depolarization that in turn activates NADPH oxidase 2 (NOX2) with subsequent production of ROS. The report by Browning *et al.* presents the effect of cessation of shear on long-term vascular adaptation *in vitro* and *in vivo* and demonstrates that ROS generated with stop of flow is an angiogenic signal. With femoral artery ligation as a model of altered shear, revascularization was high in wild-type mice and was significantly decreased in mice when cellular generation of ROS was prevented. To expand these observations, the Forum presents eight additional comprehensive reviews of previous publications.

A critical question for mechanotransduction studies relates to the direct effect of mechanical stress on cellular components. To probe this issue at a basic level, the first review in this Forum presents a novel method for measuring mechanosensitivity of individual proteins. Guo *et al.* describe the use of Forster resonance energy transfer (FRET) as a tool to assess the mechanosensitivity of specific domains within a larger protein. A FRET pair inserted into one of the domains of a protein creates a genetically encoded cassette that can be integrated into the protein structure; an increased or reduced FRET signal indicates decreased or increased interaction between the donor and the acceptor. This technique is particularly relevant to study cytoskeletal, membrane, and nuclear proteins that undergo conformational changes at the molecular level.

The transduction pathway for converting a mechanical signal into a chemical signal is another major focus of this Forum. The review by Martinac *et al.* explores this issue in unicellular organisms. Indeed, bacteria have evolved mechanosensitive (MS) ion channel proteins to cope with the cell stresses associated with fluctuations in osmolarity of the environment. Thus, when the bacterial cell membrane is stretched under excessive turgor resulting from hypo-osmotic shock, these MS channels open to release water, thereby restoring normal cellular turgor and preventing cell lysis. In their review on these channels, Martinac *et al.* discuss the use of patch clamp technology on giant spheroplasts (bacterial membrane preparations) to establish a correlation between physical forces (tension) and the alteration of the various structural domains of MS channels. These alterations drive channel opening and closure without the requirement for any other cellular components, such as the cytoskeleton or extracellular matrix. The change in turgor associated with osmotic changes is clearly a mechanical stress, although it may represent a special circumstance where the cell membrane is actually "stretched" due to an increase in intracellular pressure.

Ion channels also have been demonstrated to play an important role in mechanotransduction in mammalian cells. Based on our current understanding, it seems likely that the response of ion channels is regulated by structure and conformation of their protein components and that redox-mediated modifications of their structure may modulate those responses. Yang *et al.* discuss how the structural and functional changes in ion channels that are brought about by oxidative stress alter channel activity. Redox-dependent modifications of channel proteins include oxidation, S-nitrosylation, and glutathionylation. In particular, targeting the thiol group of cysteine residues (S-glutathionylation) in channel proteins has emerged as an essential regulator of downstream signaling (4). As a mechanotransduction cascade initiated by ion channels would hinge on channel activation/inactivation, redox status of the cell can affect the magnitude of the response to altered flow. An example described by Yang *et al.* is the K_{ATP} channel that is inhibited in oxidative stress conditions *via* S-glutathionylation of its Kir subunit.

The role of ion channels, with a major focus on endothelial K_{ATP} channels as an early responder to altered shear stress, is discussed in detail in the review by Chatterjee and Fisher. These channels appear to be responsible for the initial response to altered mechanical stress in the mammalian pulmonary microvasculature. The current concept postulates that this channel is maintained in its open conformation by normal shear in the microcirculation. An increase in shear results in hyperpolarization of the cell membrane, whereas decreased shear results in depolarization. These alterations in membrane potential are the primary transducer of the forces sensed by the cell, thereby initiating downstream signaling events. An intriguing question concerns the identity of the initial sensor of shear that then leads to the modulation of ion channel activity. Recent work on endothelium suggests that altered shear is sensed by a mechanosensory complex consisting of PECAM, VEGF, and VE-cadherin (8). PECAM, and possibly the other elements of the complex, is localized in caveolae (6), and these organelles are required for shear sensing (5). Borrowing a term previously used for studies of bone (1), Chatterjee and Fisher have called this mechanosensory complex the "mechanosome." Additional work is required to understand fully the significance of this imputed force-sensing complex. The review by Chatterjee and Fisher also emphasizes the role of NOX2 in the generation of ROS in response to altered endothelial shear stress in models of laminar flow. Endothelial cells from NOX2 null mice fail to generate ROS with stop of flow (unlike the wild

type) and fail to show downstream physiological responses, emphasizing the importance of this enzyme system.

While NOX2 has a major role in the endothelial response to altered shear stress, Brandes *et al.* describe how application of force on cells results in the activation of several different NOX enzymes. The mammalian NOX family constitutes seven different but related enzymes that may be activated *via* various mediators, including Rac1 and PIP3 and several G-protein-coupled receptors. Brandes *et al.* posit that although the upstream pathway for transduction of the stimulus is variable, all eventually translate into NOX activation. Besides shear experienced by endothelial cells, blood flow generates cyclic stretch that is experienced by vascular smooth muscle cells that underlie the endothelium. By showcasing studies where ROS scavengers and inhibitors of NOX activity prevent stretch and strain-induced differentiation, Brandes *et al.* show that NOX activity is the link between mechanosensing of a physical force and the cellular response to that force. ROS derived from NOX in response to stretch regulate intracellular calcium in skeletal muscle; in cardiac myocytes, stretch leads to NOX2 activation and ROS formation in close proximity to the ryanodine receptor, which then facilitates calcium signaling. Further discussion of the link between NOX enzymes and the cellular responses in endothelial cells and myocytes is discussed in the reviews by Raaz *et al.* and Ward *et al.*

The review by Raaz *et al.* presents evidence that the exposure of endothelial cells to oscillatory or disturbed *versus* laminar flow causes differential gene expression, which is NOX dependent. These different flow patterns have been shown to correlate with disease processes. *In vitro*, unidirectional laminar shear stress induces gene transcription profiles that are characteristic of the flow-adapted state. Oscillatory flow induces an expression profile that is pro-atherogenic through potentiation of vascular inflammation while disturbed flow initiates endothelial cell and smooth muscle hypertrophy/proliferation/apoptosis with matrix remodeling that characterizes many other vascular pathologies. Endothelial cells exposed to either oscillatory or disturbed flow generate ROS in great excess compared with cells exposed to laminar flow. Raaz *et al.* propose that the increased production of ROS accounts for the pathophysiology of the response to abnormal flow patterns. While correlation of vascular pathologies with areas of disturbed flow has been made from analysis of flow patterns and gene expression *in vivo* (7), the proposed mechanisms related to altered cell signaling are based predominantly on *in vitro* studies. However, these latter studies lack strict physiological controls since disturbed flow *in vivo* generally occurs on a background of endothelial cells that have been adapted to laminar flow rather than cells exposed to no flow at all. A crucial issue is whether the response going from “no flow” to “disturbed flow” as utilized with *in vitro* studies is similar to the response going from “laminar flow” to “disturbed flow” as might occur *in vivo*. That is not a trivial question since it is well accepted that adaptation to laminar flow results in major changes in gene expression (2, 3, 9). Furthermore, the response to disturbed flow generally has been measured over a relatively short period and represents the response to an acute change in the flow pattern. It is not yet clear how these acute changes with disturbed flow reflect the response of cells that have been exposed to disturbed flow over a longer term, that

is, in cells that might be adapted to disturbed flow. Gene expression studies reported in the review by Raaz *et al.* and previously (2) suggest differences in the adaptation state to the different flow patterns. These important questions could be answered by relatively simple experiments that have not yet been done using flow-adapted cells.

Clearly, ROS are pivotal for the physiological response to the mechanical signal in a broad spectrum of mechanosensitive cells. Ca^{2+} represents another intracellular messenger that is critical for many of the responses. The review by Ward *et al.* links these two intracellular messengers. Measurements of intracellular Ca^{2+} have shown a significant increase with shear stress in endothelial cells and with mechanical stretch in skeletal and cardiac muscle cells as discussed in the reviews of Chatterjee and Fisher and Ward *et al.* Different mechanisms for Ca^{2+} elevation associated with mechanotransduction have been described for these different cell types. In pulmonary microvascular endothelium, Ca^{2+} entry from the extracellular medium occurs through T-type voltage-gated Ca^{2+} channels that are activated by partial depolarization of the cell membrane following stop of flow. In cardiac muscle, the increased cytosolic Ca^{2+} is due to Ca^{2+} release from the endoplasmic reticulum *via* the ryanodine receptors. In skeletal muscle, increased cytoplasmic Ca^{2+} is due to Ca^{2+} influx through the sarcolemmal transient receptor potential channels. Although the precise mechanism(s) has not been identified, these Ca^{2+} channels in heart and skeletal muscle may be “sensitized” by ROS or by an increase in the oxidation state of the cell so that the Ca^{2+} influx is potentiated in the presence of oxidant stress.

Bone is another tissue where mechanosignaling is crucial for maintaining structural integrity, as discussed in the review by Knapik *et al.* Forces associated with mechanical loads activate signaling that regulates bone formation and resorption. Bone senses mechanical stimuli *via* the extensively interconnected networks of cells in the bone multicellular unit that is made up of osteocytes, osteoblasts, and osteoclasts, all of which are interconnected *via* intricate cellular networks designed to perceive and transmit mechanical stimuli and facilitate communication between cells within the bone. Excessive mechanical forces induce inflammation *via* the activation of the NF- κ B signaling cascade. Some of the responses to altered compressive forces are mediated by the release of NO. Although a possible central role for ROS in signaling has not yet been evaluated in bone cells, an increase in NF- κ B activity with increased loading clearly points in that direction.

This Forum has specifically reviewed mechanotransduction in endothelium, muscle (smooth, skeletal, cardiac), and bone as examples of cells or tissue where mechanical forces have an important physiological role. From perusal of these Forum articles, a clear pattern for mechanotransduction across a broad spectrum of cell types emerges. Mechanosensing and signal transduction act to maintain many elements of homeostasis, growth, and development while excessive or aberrant mechanosignaling can result in organ pathology. The reviews in this Forum point to an important role for NADPH oxidases (predominantly NOX2 but other NOXs as well) as pivotal for the propagation of the signal associated with the mechanotransduction cascade. Thus, mechanotransduction represents a prime example of the importance of ROS-mediated signaling in cell function.

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Abbreviations Used

FRET = Forster resonance energy transfer
MS = mechanosensitive
NOX2 = NADPH oxidase 2
ROS = reactive oxygen species