

## RhoA goes GLOBAL

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**M**echanical forces influence nearly all aspects of biology. Cells are equipped with numerous mechanosensitive proteins that activate various signaling cascades in response to mechanical cues from their environment. Much interest lies in understanding how cells respond to external stresses. A number of studies have highlighted the coordination of mechanical and chemical signaling cascades downstream of integrins. In recent years, the study of mechanotransduction has expanded to other mechanosensitive adhesion receptors, such as platelet endothelial cell adhesion molecule-1 (PECAM-1). This commentary will highlight our current understanding of integrin and PECAM-1-mediated mechanotransduction and expand on the observation that a localized mechanical stress can elicit a global mechanosignaling response.

### Introduction

Mechanotransduction, the process by which cells convert mechanical force into biochemical signaling events, is central to nearly all aspects of biology.<sup>1</sup> Mechanical forces regulate cell behavior and function during all stages of life and are central to a number of physiological and pathological processes. Therefore, a great deal of interest lies in how cells sense and respond to mechanical cues from their environment. Cells are decorated with numerous mechanosensitive proteins that work in concert to regulate the cellular response to mechanical stimuli such as stretching, shear stress, or extracellular matrix (ECM) rigidity. In addition to external forces, cells also generate their own forces (via actomyosin-based contractility) that regulate adhesion and

maintenance of cell-cell junctions.<sup>2,3</sup> A model of tensegrity has been proposed to explain how the cell coordinates internal and external stresses. This model defines the cytoskeleton as a series of interconnected compression-resistant structures that are surrounded by tensional elements. Organization of the cytoskeleton creates internal tension to provide cellular structure and support.<sup>4</sup> Application of force to the entire structure results in rearrangement of cytoskeletal elements without loss of tension. Furthermore, redistribution of cytoskeletal elements also allows force to be transmitted throughout the cell to other mechanosensitive proteins.

The best characterized mechanosensitive proteins to date are integrins: transmembrane proteins that couple the internal actin cytoskeleton to the ECM. Several studies have revealed that cells respond to exogenous force on integrins by strengthening or stiffening adhesions to resist increased tensile strain.<sup>5-7</sup> Applied force on integrins involves recruitment of vinculin and growth of focal adhesions in the direction of applied force.<sup>8</sup> Adaptive stiffening also requires coordination of mechanically activated signaling cascades, including activation of RhoA and its effectors, which ultimately mediate local changes in focal adhesion growth and actomyosin contractility.<sup>5-7</sup> In recent years, researchers have begun to probe other mechanosensitive proteins, such as platelet endothelial cell adhesion molecule-1 (PECAM-1). PECAM-1 is expressed in only a subset of cells, including endothelial cells, where it plays an important role in transducing shear stress due to blood flow into various biochemical signals.<sup>9</sup> Mechanical probing of PECAM-1 has revealed that adaptive stiffening or strengthening in response to

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**Abbreviations:** PECAM-1, platelet endothelial cell adhesion molecule-1; ECM, extracellular matrix; RGD, Arg-Gly-Asp; PI3K, phosphoinositide 3-kinase; FN, fibronectin; FAK, focal adhesion kinase

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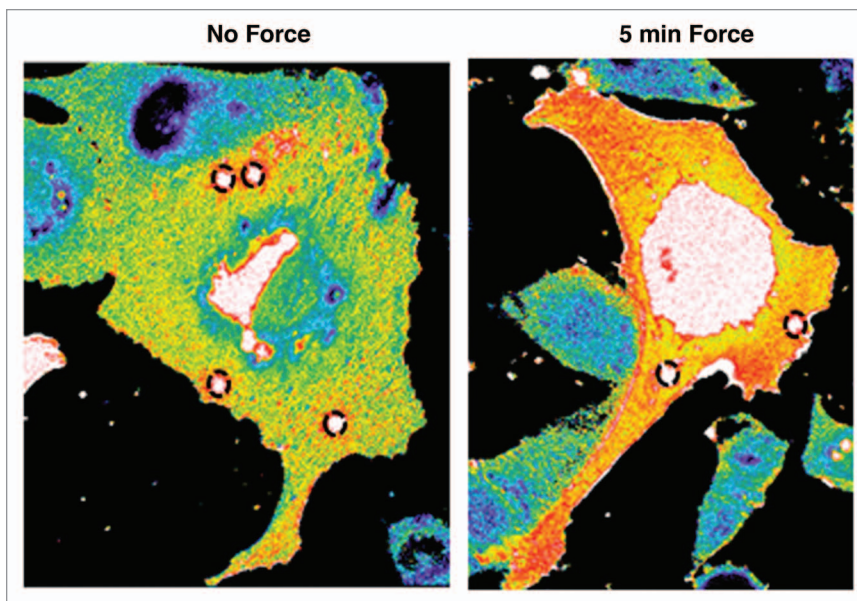
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exogenous force is not specific to integrins, but may be a commonality shared by multiple mechanosensitive proteins. However, recent work has shown that while common sets of molecular mechanisms exist, specialized systems display unique mechanotransduction responses. This commentary will compare and contrast known mechanotransduction pathways in response to applied tension to integrins and PECAM-1.

### Common Mechanotransduction Responses

A number of experimental approaches to apply tension on integrins, including optical or magnetic tweezers and electromagnetic microneedles, have shown that cells can sense the applied tension and respond by strengthening their cytoskeletal linkages to oppose the force.<sup>6,7,10-12</sup> Similar to integrins, application of tensional forces on anti-PECAM-1-coated beads bound to endothelial cells induces a stiffening response that also depends on the actin cytoskeleton.

In addition to adaptive stiffening, several other mechanosensitive responses are also shared between PECAM-1 and integrins. Force application on either adhesion receptor results in RhoA activation through the same two GEFs, GEF-H1 and LARG.<sup>12,13</sup> Interestingly, RhoA activation in response to tensional force on PECAM-1 is integrin-dependent, as inhibition of new integrin-ECM connections quenches force-induced RhoA activity.<sup>13</sup> This integrin-dependent RhoA activation might explain the shared GEFs in both systems. Importantly, RhoA-mediated signaling is required for adaptive stiffening downstream of both integrins and PECAM-1, as inhibition of RhoA, its GEFs, or its effectors abolishes the force-dependent stiffening response. Finally, tension on either integrins or PECAM-1 also results in growth of focal adhesions, which mechanically couple the internal actin cytoskeleton to the extracellular matrix.<sup>8,13</sup> Indeed, the best-defined response to applied tension on integrins is reinforcement of the focal adhesions to counteract the exogenously applied force.<sup>5</sup> Similarly, force on PECAM-1 results in growth of focal adhesions. However, in



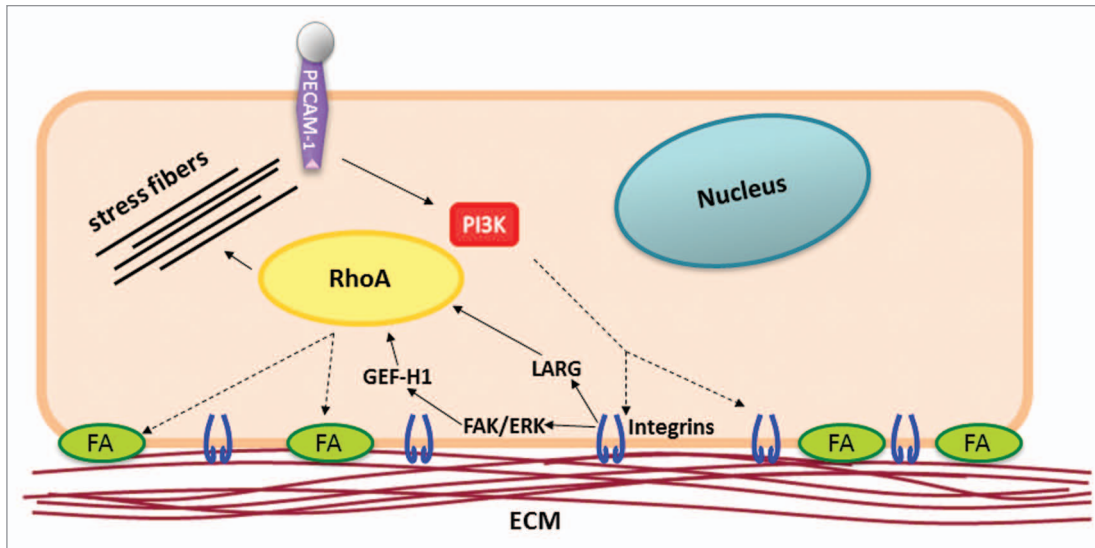
**Figure 1.** Localized tension on PECAM-1 elicits global RhoA activation. Endothelial cells expressing the RhoA biosensor were incubated with anti-PECAM-1-coated beads and subjected to force with a permanent ceramic magnet for the indicated times. Cells were fixed and analyzed for FRET. Autofluorescent beads are highlighted in black dotted circles.

contrast to the local adhesion growth observed in response to tension on integrins, PECAM-1-mediated adhesion growth is a global phenomenon. This leads us to the important differences in mechanotransduction cascades between these two cell adhesion molecules that will be discussed below.

### Differences in Mechanotransduction Pathways

Several differences exist when comparing the cellular response to force application on integrins vs. PECAM-1. Closer examination of some of the shared responses, such as adaptive stiffening or RhoA activation, also reveal some notable differences in the cellular responses downstream of each receptor. While mechanical stimulation of either adhesion receptor results in adaptive stiffening, the time course of the response varies between the two proteins. Tension applied to fibronectin (FN)-binding integrins results in an immediate stiffening response, as differences in cellular stiffness can be detected immediately following force application. Conversely, while force application on PECAM-1 also elicits adaptive stiffening, the response is delayed and is detectable on a minute,

rather than second timescale. This same trend is also observed when taking a closer look at the time course of RhoA activation. Force application on integrins results in an increase in RhoA activity in as little as 1 min. In contrast, force-induced RhoA activation downstream of PECAM-1 does not increase until 5 min of force. Therefore, it is possible that the delay in the PECAM-1-mediated adaptive stiffening response is a reflection of delayed RhoA activation. Differences in the time course of these events may be attributed to differences in mechanisms of signal transduction. Mechanosensitive signaling pathways may be chemically or mechanically propagated throughout the cell, via diffusion of small chemical messengers or mechanically transmitted through cytoskeletal filaments, respectively. Indeed, mechanical signals can be propagated at a remarkable speed of 30 m/s, whereas biochemical signals diffuse at a mere rate of 2  $\mu\text{m/s}$ .<sup>14</sup> Given integrins' intimate association with the cytoskeleton, it is possible that integrin-mediated mechanotransduction relies more heavily on mechanical transmission of the signaling cascade, while PECAM-1-mediated mechanotransduction is more chemically-dependent in nature.



**Figure 2.** Model of PECAM-1-mediated mechanotransduction. Force-dependent activation of PI3K downstream of PECAM-1 promotes global activation of integrins, cell-wide activation of RhoA, and global growth of focal adhesions. Cell-wide propagation of the signaling cascade facilitates adaptive stiffening and strengthening of adhesions, which function to resist the applied force. Adapted from Collins et al.<sup>13</sup>

Perhaps the most profound difference between integrin and PECAM-1-mediated mechanosignaling involves the spatial organization of the response. Several studies have revealed that integrin-mediated adaptive stiffening response is mediated, in part, by localized recruitment of focal adhesion proteins (such as  $\beta_1$  and  $\beta_3$  integrins, talin, and vinculin), which results in a local growth of adhesions at the site of force application.<sup>8,15</sup> Similarly, force application on integrins induces localized recruitment of LARG and GEF-H1 to the adhesion complex. Furthermore, mRNA and ribosomes also specifically localize to focal adhesions that form under adherent FN-coated microbeads.<sup>16</sup> Surprisingly, our work has revealed that tension on PECAM-1 results in a remarkable cell-wide, or global, mechanotransduction response, as evidenced by a cell-wide increase in focal adhesion growth. This result is especially intriguing, as it is the first documented evidence of a global mechanotransduction event elicited by a localized force. While studies from Ning Wang's group have reported Src and Rac activation at remote sites away from stress application with RGD-coated beads,<sup>14,17,18</sup> neither signal displayed cell-wide distribution. Nevertheless, it has been shown that global, cell-wide changes in cytoskeletal structure and mechanics can regulate mechanotransduction, such

that the mechanotransduction response is governed by global mechanical cues, including isometric tension (pre-stress) within the cytoskeleton.<sup>18,19</sup>

### Globalization of Mechanotransduction

How does a localized force on PECAM-1 elicit a global response? Phosphoinositide 3-kinase (PI3K) is rapidly activated following application of tension on PECAM-1. Activation of PI3K results in the production of freely diffusible phosphoinositides [such as  $PI(3,4,5)P_3$ ] that act as second messengers to propagate signaling cascades throughout the cell. Experimental evidence suggests that: (1) activation of PI3K, and (2) diffusion of the second messenger  $PI(3,4,5)P_3$  is required for the global response downstream of PECAM-1. Localized tension on PECAM-1 not only elicits global focal adhesion formation, but also results in global integrin and RhoA activation (Fig. 1). Interestingly, inhibition of PI3K activation, or sequestration of the  $PI(3,4,5)P_3$  messenger, abolishes the global signaling response. PI3K has been implicated in integrin activation in various contexts and cell types, although the exact mechanism of activation remains elusive.<sup>20</sup>  $PI(3,4,5)P_3$  has a documented half-life 60 sec<sup>21</sup> and a fairly

rapid diffusion rate of  $0.1-1 \mu m^2/sec$ .<sup>22</sup> Given that endothelial cells are relatively thin cells (generally less than  $5 \mu m$  in height), it is feasible that freely diffusing  $PI(3,4,5)P_3$  would be able to globally activate integrins at cellular sites proximal and distal to the site of force application. Therefore, the current model of PECAM-1-mediated mechanotransduction suggests that PI3K is rapidly activated downstream of PECAM-1, and  $PI(3,4,5)P_3$  promotes global integrin activation (Fig. 2). Integrin activation is followed by new binding to the ECM and enlargement of adhesions. Cell-wide activation of integrins elicits global activation of RhoA via the GEF-H1 and LARG pathway. This is a crucial aspect of the PECAM-1-mediated response, as global activation of the GTPase facilitates global growth of focal adhesions, which contributes to the adaptive stiffening response. This model also highlights cooperation of 2 mechanosensors in this system: PECAM-1 and integrins. Not only does PECAM-1 lead to activation of integrins, but integrin ligation with the ECM is required for force-induced RhoA activation and adaptive stiffening.

The requirement of PI3K activation and  $PI(3,4,5)P_3$  implies that chemical signaling is critical for the global nature of the PECAM-1-mediated response. However, it remains unclear if mechanical

propagation of the signal is also required for the global response. Transmission of force through tensile cytoskeletal elements can propagate mechanosensitive signaling away from sites of applied stress. For instance, previous studies probing integrins reported rapid activation of Src and Rac at remote cytoplasmic locations away from the site of mechanical stress.<sup>14,17,23</sup> However, these signals were not global in nature, but rather were confined to distinct puncta that corresponded with sites of cytoskeletal deformation. It is also important to note that integrins' physical association with the cytoskeleton has been well documented. Therefore, it is plausible that tensile cytoskeletal filaments anchored to integrins on opposing sides of the cell could facilitate transmission of a mechanical stimulus across the cell. However, PECAM-1 has not been shown to directly interact with the cytoskeleton; although indirect association via cytoplasmic interactions with  $\beta$ -catenin and  $\gamma$ -catenin have been proposed.<sup>24</sup> Furthermore, the exact of mechanism of PECAM-1-mediated mechanosensing remains unknown. Models of mechanotransduction often involve force-dependent conformational changes in proteins that permit propagation of mechanosensitive signaling cascades. For instance,  $\alpha_5\beta_1$  integrin undergoes a force-dependent conformational change,<sup>25</sup> which allows chemical crosslinking to fibronectin and facilitates recruitment and activation focal adhesion kinase (FAK). While it is appealing to hypothesize that PECAM-1-mediated mechanotransduction may be dependent on a conformational change, a force-dependent change in the protein structure has not yet been identified, and thus, the mechanism of mechanosensing remains elusive. Comprehensive studies investigating the structure of the PECAM-1 cytoplasmic tail may provide insights into the mechanisms of PECAM-1-mediated mechanosignaling and reveal the relative contributions of chemical signaling and mechanical transmission in the global mechanotransduction response.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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