EDITORIALS

The Force Awakens in the Cytoskeleton: The Saga of a Shape-Shifter

Any Trekkies can tell you stories about Odo, the shape-shifter who can transform himself from his resting state (gelatinous goo) to pretty much any shape he wants. It turns out that smooth muscle cells also possess, to some extent, this incredible talent. The ability of smooth muscle to function over a large range of lengths requires that the contractile apparatus within the muscle cells be able to generate and transmit force at extreme lengths. Previous research revealed that the structure of the contractile apparatus of smooth muscle is plastic and can be reconfigured to function optimally at any adapted cell lengths (1, 2). This adaptation process does not happen instantly after a length change; rather, the process happens following a certain sequence (3). There is evidence suggesting that in adapting to different lengths, contractile units, akin to sarcomeres in striated muscle, can be added or subtracted in series to maintain optimal overlap between actin and myosin filaments (4). This degree of malleability appears to be facilitated by labile myosin filaments that can be depolymerized or fragmented easily during the initial phase of adaptation triggered by a length change. Reformation of the filaments (likely at different locations within the contractile apparatus) occurs during the final phase of adaptation (5, 6).

Restructuring of the contractile apparatus in smooth muscle during length adaptation is not limited to reconfiguration of contractile units; the scaffold that supports the contractile units and physically links them to neighboring cells and extracellular matrix (ECM) has to undergo a similar extent of structural transformation. There is evidence suggesting that the anchoring points where contractile units are attached to dense plaques (protein structures for mechanical cell–cell or cell–ECM coupling) via actin filaments are not permanent connections; rather, the connections can be severed during muscle relaxation and reestablished at the onset of contraction, not necessarily at the same locations. Attachment of actin filaments at the focal adhesion sites involves chemical modification of many adaptor proteins, such as vinculin and paxillin. Regulation of adaptor proteins by phosphorylation mediated by various kinases through an intricate network of signaling pathways has been revealed in the last few years (7–11). In this issue of the *Journal*, Wang and colleagues (pp. 645–656) advance our knowledge about the regulation of the shape-shifting abilities of the cytoskeleton in smooth muscle (12). The authors discovered that a serine/threonine protein kinase, SLK (Ste20-like kinase), functions as a master regulator of actin polymerization, promoting force development and maintenance in airway smooth muscle cells. This force transduction is not coupled to myosin light chain phosphorylation but rather is cooperatively mediated by forming a complex with another serine/threonine kinase, Plk1 (polo-like kinase 1), which had been previously reported to regulate paxillin phosphorylation at Ser-272 (13). Because SLK does not directly catalyze paxillin phosphorylation *in vitro*, in contrast to Plk1, the authors conclude that SLK is an upstream regulator of Plk1 and promotes the structural transformation necessary for optimal cell function across a large-length scale via reattachment of actin filaments within the cytoskeleton facilitated by activation of adapter proteins.

Our understanding of the roles cytoskeletal proteins play in force transmission and generation in smooth muscle is far from complete. The relatively small extent of actin polymerization during contractile activation suggests that the polymerization may serve to facilitate only formation of focal adhesions but not formation of contractile units. This notion is consistent with the observation that in the relaxed state actin filaments outnumber myosin filaments by a factor of greater than 20 in airway smooth muscle, considering that an actin/myosin ratio of 2:1 is sufficient for full force generation (14). Another puzzle regarding the role of cytoskeletal proteins in force transmission is that the rate of force development after contractile stimulation in smooth muscle seems always to be faster than the rate of phosphorylation of cytoskeletal proteins (*see* an example in Figure 2A, Reference 12). The rate of force development should be determined by the rate-limiting process of myosin light chain phosphorylation or phosphorylation of cytoskeletal proteins (whichever is the slowest). A satisfactory explanation for the time lag in phosphorylation of cytoskeletal proteins behind that of force development is still needed.

An emerging picture depicts two distinct domains for how smooth muscle cells work. One is responsible for force generation and the other for force transmission, and each of them has its own regulatory signaling pathway. With few exceptions, such as Rho-kinase, enzymes involved in one pathway do not participate in the regulation of the other. As demonstrated by Wang and colleagues (12) and by many previous studies (7–11), disrupting the signaling pathways regulating cytoskeletal dynamics has no effect on the degree of phosphorylation of the regulatory myosin light chain, an indicator of muscle activation. Many lines of evidence indicate that cytoskeletal stiffness (a measure of the overall physical integrity of the cytoskeleton) can be separated from the muscle stiffness stemming from active binding of myosin cross-bridges to actin filaments and that from the ECM (15). The complex and compartmentalized pathways may present challenges to our understanding of regulation of smooth muscle contraction; however, they also provide opportunities for identifying drug targets for smooth muscle–related diseases. One main strategy for asthma treatment is focused on bronchodilation through the use of β_2-agonists specifically to inhibit active force generation by airway smooth muscle. With the newly gained knowledge on how cytoskeletal stiffness affects airway distensibility, new drugs could be developed in the form of inhibitors of enzymes associated with

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the regulation of cytoskeletal dynamics, such as SLK and Plk1 described by Wang and colleagues (12). ■

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