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Stretch-induced actomyosin contraction in epithelial tubes: mechanotransduction pathways for tubular homeostasis

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Abstract

Many tissues in our body have a tubular shape and are constantly exposed to various stresses. Luminal pressure imposes tension on the epithelial and myoepithelial or smooth muscle cells surrounding the lumen of the tubes. Contractile forces generated by actomyosin assemblies within these cells oppose the luminal pressure and must be calibrated to maintain tube diameter homeostasis and tissue integrity. In this review, we discuss mechanotransduction pathways that can lead from sensation of cell stretch to activation of actomyosin contractility, providing rapid mechanochemical feedback for proper tubular tissue function.

Keywords

Stretch; epithelial tubes; smooth muscle; mechanotransduction; contractility

Introduction

Tubular structures, such as blood vessels, exocrine glands, and airway passages, are a common feature of the animal body plan. In the simplest form, tubes have a central lumen that is enveloped by a layer of epithelial or endothelial cells. Physiological tubes often consist of multiple layers of cells, including myoepithelial or smooth muscle cells. Though each tubular structure in the body is anatomically distinct, they all experience external forces that influence their structure and function. Cells in tubes become stretched as a result of intraluminal pressure, either in a constant regime or cyclically, for example following inhalation and exhalation of air from the lungs. Cells also experience shear stress when fluid or air flows through the tube. The response of epithelial and endothelial cells to shear stress

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has been extensively reviewed elsewhere [1–3]. Here, we focus on the response of epithelial tubes to stretch, and in particular on signaling pathways that are initiated by stretch and lead directly to activation of actomyosin contractility.

Increasing luminal pressure acts to stretch tubular structures, which are elastic at short time scales [4], just as pumping air into the inner tube of a bicycle tire will inflate it. In contrast to a tire tube, whose passive response to stretching depends solely on its material properties, cells can actively respond by modulating their cytoskeleton (see Figure 1). Mounting a cellular response to stretch requires conversion of physical force into intracellular biochemical signals, a process named mechanotransduction [5,6]. Mechanosensing proteins directly perceive mechanical cues and are activated or inactivated in response. Signals from mechanosensors, located at sites of cell-matrix and cell-cell adhesion, the cytoskeleton and the plasma membrane, are then relayed to secondary messengers, which function through various pathways to allow cells to respond to the stress. This response could be a rapid response, such as an increase in intracellular tension or an increase in intracellular calcium concentration. Additionally, there could be a delayed response, such as signaling to the nucleus, culminating in changes in gene expression [7,8] or reorientation of the cytoskeleton and cells at an angle to the direction of stress [9–12].

The most direct response to cell stretching is an increase in intracellular tension to balance the applied force. The actomyosin contractile apparatus builds up this tension, which is essential to maintain the integrity of the cells and also of the tubular structure as a whole. Luminal pressure is countered by passive mechanical resistance and contractility of the cells surrounding the lumen, and these forces must be balanced to maintain a stable tube diameter (Figure 1). Further, a reduction in the intraluminal pressure would ultimately induce a reversal of the contracted state of the cells, resulting in homoeostasis in the relaxed state. Epithelial tube contractility is also externally regulated by hormones and neurotransmitters [13–15]. However, we would like to suggest here that mechanotransduction pathways leading directly from stretch-sensing to activation of the contractile apparatus are the most effective way to respond to external forces, and are probably employed by tubular structures *in vivo*.

The contractile apparatus in smooth muscle and in non-muscle cells is composed of arrays of actin and myosin II filaments [16]. As in skeletal muscle, ATP hydrolysis powers the inward sliding of actin filaments towards bipolar filaments of myosin motors [17]. However, in contrast with the highly organized and stable sarcomere structure of skeletal muscle, actomyosin in smooth muscle and in non-muscle cells is more loosely organized into sarcomere-like stress fibers that can assemble and disassemble within minutes. Actin polymerization from its free monomeric form (G-actin) to filamentous actin (F-actin) is catalyzed by actin nucleation and elongation factors, such as formins and the Arp2/3 complex [18,19]. *In vitro*, as airway smooth muscle cells are stretched, an increase in the ratio of F-actin:G-actin is observed [20]. Further remodeling of actin filaments by actin bundling and crosslinking proteins gives rise to a network of filamentous actin. *In vivo*, smooth muscle actin isoform ACTA2$^{-/-}$ mice show impaired mammary gland contraction and milk ejection, highlighting the role of actin in tube contractility [21]. Myosin II consists of two heavy chains, two regulatory light chains and two essential light chains.

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Phosphorylation of two residues on the regulatory myosin light chain, MLC, is required for the activity of myosin [22]. Myosin light chain kinase, MLCK, phosphorylates MLC, promoting contractility. Conversely, myosin light chain phosphatase, MLCP, inhibits contractility by dephosphorylation of MLC [23]. Thus, contractility can be controlled at two main levels: regulation of F-actin polymerization/remodeling and regulation of MLC phosphorylation.

In the following sections, we highlight three major signaling pathways that can be activated by cell stretching and lead to actin polymerization and/or myosin phosphorylation and hence to actomyosin contractility. A schematic representation of these pathways is shown in Figure 2.

Integrin-mediated signaling

Integrins are heterodimeric transmembrane receptors [24]. They bind extracellular matrix (ECM) ligands with their extracellular head domains and bind to multiple intracellular proteins through their short cytoplasmic tails. It is through integrins that the ECM is connected to the intracellular contractile machinery. Cyclically stretched endothelial cells fail to reorient their cytoskeleton upon inhibition of integrin function [25], highlighting the importance of integrins in transducing mechanical cues. In addition, cyclically stretched endothelial cells show an upregulation of β3-integrin [26]. *In vivo*, knockout of β1-integrin in mice urothelium affects mechanosensing of urinary bladder filling (stretch), resulting in incontinence [27]. Additionally, integrins may also have a role in completion of the contraction/relaxation cycle *in vivo*, as a conditional mutant of integrin α3β1 in the mammary myoepithelial cells results in hypercontractility of the alveoli in the mammary gland [28].

Integrins can exist in an inactive or in an active state and their activation can be brought about by internal or external factors [29]. Inside-out signaling refers to the activation of integrins following the binding of talin or kindlin to the cytoplasmic tail [30,31]. On the other hand, outside-in integrin signaling occurs when integrins are activated by binding of a ligand within the ECM. Activation of integrins induces a conformational change in their structure, leading to clustering and a change from a low affinity state to a high affinity state, which forms focal adhesion sites [32]. In the high affinity activated state, clustering of integrins promotes focal adhesion kinase (FAK) activity, which in turn recruits Src-family kinase to adhesion sites [33]. As seen in umbilical vein endothelial cells, stretch promotes phosphorylation of focal adhesion kinase (FAK) [34], which in turn interacts with focal adhesion proteins, such as paxillin [35]. Additionally, cyclic stretching promotes increased expression and enhanced recruitment of focal adhesion components, such as paxillin and vinculin, at focal adhesion sites, as is seen in stretched smooth muscle cells [36,37]. In parallel, force applied to cells induces conformational changes in proteins, such as that of talin. This change in conformation exposes cryptic binding sites for other signaling/adapter proteins, such as that of vinculin on stretched talin [38].

Integrin-mediated signaling functions to reinforce the cytoskeletal machinery. One way it does this is through the LIM domain protein zyxin. Zyxin localizes to focal adhesion sites

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[39] and is known to bind to both the actin crosslinking protein, α-actinin and the actin polymerization protein, Ena/VASP [40–42]. Upon cellular stretch, zyxin, along with VASP (in a zyxin dependent manner), mobilizes from focal adhesion sites to stress fibers and thus contributes to actin remodeling [43,44]. Interestingly, zyxin is highly expressed in cells of the vasculature, lungs and urinary bladder, which experience stretch forces *in vivo* [45]. *In vivo*, zyxin is not essential for mechanotransduction, as zyxin null mice are viable [46]. However, vascular smooth muscle cells from zyxin null mice show reduced contractility with a poorly organized actin network [47].

Another way integrin-mediated signaling reinforces the actin cytoskeleton is through recruitment and activation of Rho guanine nucleotide exchange factors (GEFs), which stimulate RhoA signaling (discussed below).

Calcium-mediated signaling

Calcium plays a central role in cell contractility, in non-muscle cells, primarily through the calcium-calmodulin dependent activation of myosin light chain kinase, MLCK [48]. MLCK phosphorylates the myosin regulatory light chains, resulting in myosin activation, bipolar filament formation, and contraction of the actomyosin cytoskeleton [49].

One important source of calcium is through ion channels at the plasma membrane, which allow for influx of calcium into the cell from the extracellular environment. Mechanical stimulation, such as stretch, may activate ion channels. Influx of calcium through ion channels is essential for a mechanoresponse to stretch. For example, inhibition of stretch-activated calcium channels with gadolinium chloride almost completely abolishes calcium influx and subsequent cytoskeletal remodeling in cultured endothelial cells [50]. Possible mechanisms include direct mechanosensing (change in conformation of the channel under tension), activation through a tethered cytoskeletal element or activation by an upstream protein in the pathway. Ion channels expressed at the endothelial cell membrane have been studied extensively and among them, the transient receptor potential vanilloid (TRPV) superfamily of channels play an important role in mechanosignaling [25]. Inhibition of TRPV4 function in capillary endothelial cells, either by using an inhibitor molecule or through specific siRNA treatment, inhibited influx of calcium and subsequent reorientation of cells to stretch [25]. Similarly, TRPV1 and TRPV4, expressed in the urothelium, have been proposed to sense bladder distension and are required for normal bladder function [51–53]. Other studies have shown a crosstalk between integrins and TRPV channels with a mutual dependency for activation [25,54]. Further, TRPV4 associates with α-catenin at adherens junctions [55], F-actin [56], and non-muscle myosin IIa [57] in different cell types, consistent with a role in mechanotransduction.

Another major source of calcium is calcium stored in the endoplasmic reticulum. Calcium entry from both intracellular and extracellular sources can be activated by G-protein coupled receptors (GPCRs). GPCRs are integral membrane proteins, expressed at the cell surface, that can be activated by the action of agonists, such as activation of the GPCR oxytocin receptor upon binding of oxytocin [15]. Importantly, it has been suggested that the GPCR

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angiotensin II type 1, AT1 receptor in cardiac myocytes is directly mechanosensitive [58,59], but identification of other mechanosensitive GPCRs is still elusive.

Among other effectors, GPCR activates phospholipase C (PLC) leading to the release of calcium from internal stores. PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) to produce the second messengers, diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). IP_3 diffuses into the cytoplasm and binds to IP_3 receptors at the endoplasmic reticulum, promoting the release of the intracellular stored calcium pool. Additionally, DAG can activate ion channels at the plasma membrane and allow for influx of extracellular calcium [60,61].

In connected cells, propagation of intercellular calcium waves through gap junctions is important for coordinated tissue responses to mechanical input. For example, gap junctions are required for the propagation of calcium waves through human retinal pigment epithelium in response to mechanical stimulation [62], and the gap junction protein innexin-12 is required for propagation of calcium waves in response to the stretch of oocyte entry in the *C. elegans* spermatheca [63], a contractile myoepthelial tissue in the nematode's reproductive system. Although little is known about the response of gap junctions to mechanical perturbation in epithelial tissues, evidence from cardiomyocytes and bone cells suggests that the composition and localization of gap junctions is responsive to mechanical stimulation [64–67]. If a similar mechanism is operational in epithelial cells, gap junctions could, in addition to transmitting the signal between cells, contribute to the spatio-temporal regulation of mechanically triggered calcium signaling.

Filamin, an actin-binding and mechanically-sensitive scaffold protein, also contributes to contractility through crosslinking the actin cytoskeleton and by initiating calcium influx [68]. For example, a reduction of function mutant of filamin in the *C. elegans* spermatheca has a significantly disrupted actin cytoskeleton and fails to initiate calcium waves, resulting in impaired spermathecal contractile activity [63,69]. Similarly, a deletion mutant of filamin-A in smooth muscle cells in mice results in disruption of calcium influx upon increase in intraluminal pressure and consequently impairs myogenic behaviour by lowering of basal arterial pressure [70]. Filamin anchors many transmembrane proteins, including the CaR extracellular calcium receptor, to the actin cytoskeleton [71]. While it has not been directly demonstrated, filamin tethering of stretch-gated ion channels to the cytoskeleton might enable them to be activated by actomyosin contraction. Although these interactions are stabilizing or activating, the interaction between filamin and channels, such as the polycystin TRP-type channel in human renal epithelial cells, can also be inhibitory [72].

RhoA-mediated signaling

The small G protein RhoA is a key regulator of contractility [73]. RhoA switches between an active GTP-bound state and an inactive GDP-bound state. In its inactive state, RhoA is retained in the cytosol, associated with a GDP dissociation inhibitor (GDI). GTPase activating proteins (GAPs) inactivate RhoA by promoting hydrolysis of GTP (bound to RhoA) to GDP. Activation of RhoA is mediated by guanine nucleotide exchange factors (GEFs) [74]. Once activated and localized to the membrane, RhoA can signal to downstream

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pathways to respond to stretch. The pivotal role of RhoA in mechanosignaling is highlighted in cultured airway smooth muscle cells expressing a dominant negative mutant of RhoA wherein the cells failed to reorient in response to stretch [20].

We recently identified a RhoGAP protein, SPV-1, that regulates contractility of the *C. elegans* spermatheca [75]. Contraction of the spermatheca is driven by calcium-calmodulin activation of myosin light chain kinase and RHO-1 activation of Rho kinase (ROCK), which leads to the phosphorylation and activation of non-muscle myosin II [63,76]. In addition to its GAP domain, SPV-1 also posseses an F-BAR domain, which binds curved membranes. In a relaxed membrane state, SPV-1 binds to the membrane through its F-BAR domain and effectively prevents spermatheca contractility by inhibiting RhoA activity through its GAP domain. Upon membrane stretch (oocyte entry into spermatheca), SPV-1 dissociates from the membrane, with subsequent recruitment of active RhoA that induces contractility of the spermatheca [75].

GEFs are often recruited and/or activated by upstream effectors, such as integral membrane receptors, including integrins and Gq-coupled GPCRs, and kinases. For example, upon activation of integrins by applying force on fibroblast cells with magnetic beads, the GEFs GEF-H1 (ARHGEF2) and LARG (ARHGEF12) are activated and recruited to focal adhesion sites [77]. These GEFs activate RhoA, also present at focal adhesion sites, and thus promote contractility. Likewise, cyclic stretch of HUVECs showed enrichment of the GEF Solo (ARHGEF40) at adherens junctions [78]. Similar to GEF-H1 and LARG, Solo activates RhoA, though at cell-cell contact sites, promoting contracility.

RhoA promotes contractility by regulating both actin remodeling and myosin phosphorylation status. Binding of RhoA induces a conformational change in Rho kinase (ROCK) [79,80]. This conformational change of ROCK results in its autophosphorylation and activation. ROCK activation is important for contractile response, as upon inhibition of ROCK activity with the ROCK inhibitor Y27632, the basal tone of cyclically stretched isolated human bronchi is significantly affected [81]. Once activated, ROCK phosphorylates MLC [82], enabling myosin binding to actin and contraction [83]. Also, ROCK phosphorylates the myosin binding subunit of MLCP, relieving its inhibitory effect on MLC [84]. As a consequence, MLC is now maintained in a phosphorylated active status, enabling contraction. Activated ROCK also affects actin stability by stimulation of LIM kinase which phosphorylates and inhibits the actin-severing protein, cofilin [85]. In parallel, activated RhoA also activates the formin protein, mDia1 [86], driving polymerization of F-actin that can incorporate into stress fibers. As a result of formin activation and cofilin inhibition, filamentous actin structures are stabilized and together with the activity of myosin can increase cellular tension/contractility.

It has also been suggested that actin filaments themselves can sense tension [87]: tensed actin filaments show an enhanced affinity for myosin [88] while hindering cofilin binding [89]. Both cell culture [90–92] and *in vivo* work [93–96], for example, in the epithelial tubes of *Drosophila* trachea and *C. elegans* spermatheca, have shown that cell stretch and contraction influences actin organization, suggesting reorganization of the actin cytoskeleton is an important cellular mechanism for adaptation to the mechanical microenvironment.

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Stress fibers, contractile actomyosin bundles, are common among cells exposed to physical stress and have been well-studied within the context of cell migration [97–102]. However, much less is known about regulation of contractile stress fiber-like structures in epithelial tubes.

Conclusions and future directions

In this review, we have highlighted pathways through which cell contractility can be directly activated in response to stretching forces. Cells *in vivo*, particularly those in tubular structures, commonly experience stretching forces. However, very few studies have addressed the cellular responses to stretching *in vivo*. This is partly because of technical challenges and a shortage of amenable *in vivo* models. Additionally, the fact that multiple mechanical cues influence cells *in vivo* and that multiple biochemical pathways also regulate contractility, further complicates the issues at hand. In the future, advanced imaging techniques, novel biosensors and use of conditional mutants should further enhance our understanding of cellular contractile responses to stretch within tubular tissues *in vivo*. Further studies are also required to better understand the assembly and organization of the contractile machinery within *in vivo* tubes. Basic questions include how actin bundles are aligned within cells and what determines their dominant orientation, which proteins contribute to the development and maintenance of stress fiber-like structures and how forces might contribute to these processes.

We expect that tube homeostasis is achieved through a combination of both short term and long term regulation of the actomyosin cytoskeleton and that mechanotransduction processes play a role in both, in combination with biochemical signals such as hormones and neuropeptides. Understanding the implications of stretch on cells is of clinical importance, because errors in the mechanotransduction process may impact various disease states. For example, one of the underlying causes of asthma is excessive hypercontractility of the bronchial airways. As another example, an increase in vascular stiffness, such as by deposition of collagen, results in increased cellular tension and consequently high blood pressure. Thus, knowledge of the mechanotransduction pathways involved in regulating tubular contractility might suggest novel therapeutic targets.

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Abbreviations

ATP	adenosine triphosphate
DAG	diacylglycerol
ECM	extracellular matrix
FAK	focal adhesion kinase
GAP	GTPase activating protein
GDI	guanine dissociation factor
GEF	guanine nucleotide exchange factor
GPCR	G protein-coupled receptors
HUVEC	Human Umbilical Vein Endothelial Cells
IP_3	inositol 1,4,5-trisphosphate
MLC	myosin light chain
MLCK	myosin light chain kinase
MLCP	myosin light chain phosphatase

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PIP_2	phosphatidylinositol 4,5-bisphosphate
PLC	phospholipase C
ROCK	Rho-associated protein kinase
TRPV	transient receptor potential vanilloid.

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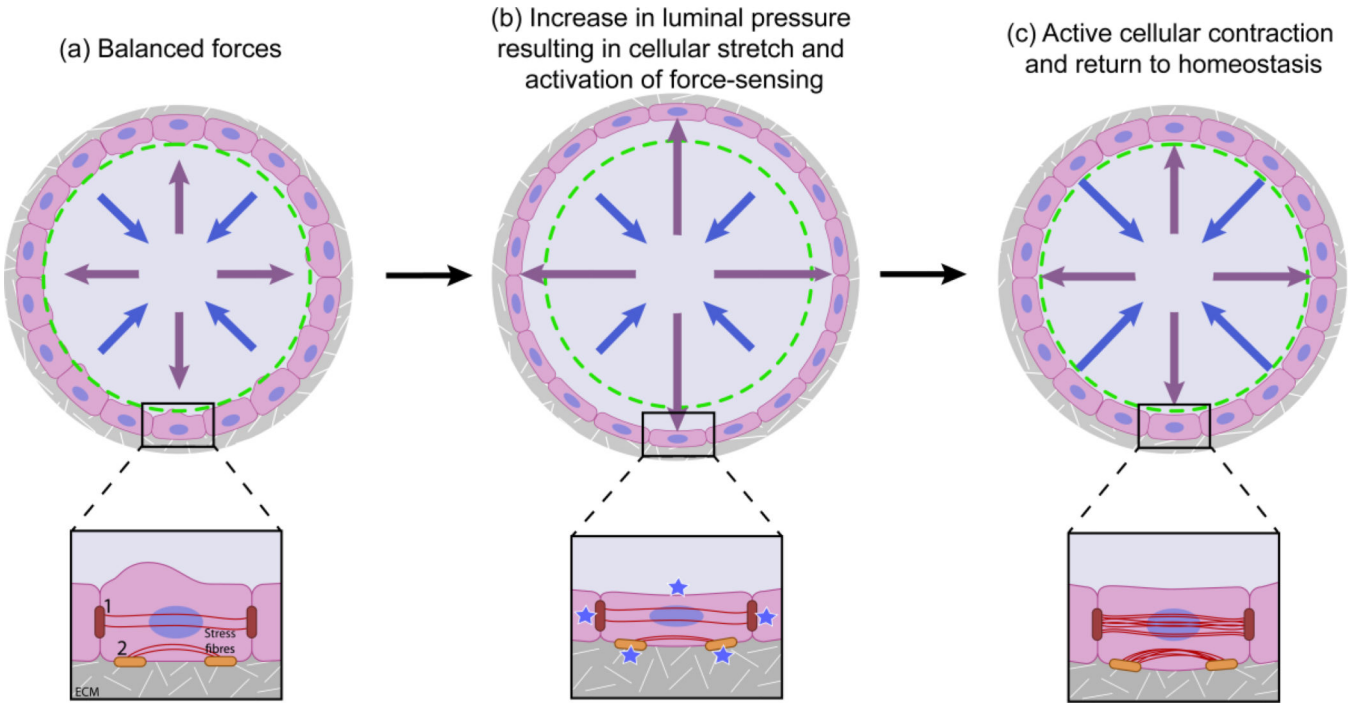

Figure 1. A balance between luminal pressue and cellular contractility determines the diameter of epithelial tubes

Schematic depiction of the force balance within epithelial tubes (top panel) and within individual cells of tubes (bottom panel). (a) In a state of homeostasis, the outward luminal pressure (purple arrows) is balanced by the mechanical properties of the cells (blue arrows). Cells are attached to their neighbors through cell-cell junctions (marked as 1) and to the ECM through integrin adhesions (marked as 2). (b) Upon an increase in intraluminal pressure, as upon inhalation of air in lung alveoi, the cells around the tube stretch and force-sensing occurs at the membrane, integrin adhesions and cell-cell junctions (blue stars). (c) In response to force-sensing at integrin adhesions and cell-cell junctions, the cells increase their intracellular tension by active contraction of the actin cytoskeleton (red lines), culminating in return to a state of homeostasis within the tube.

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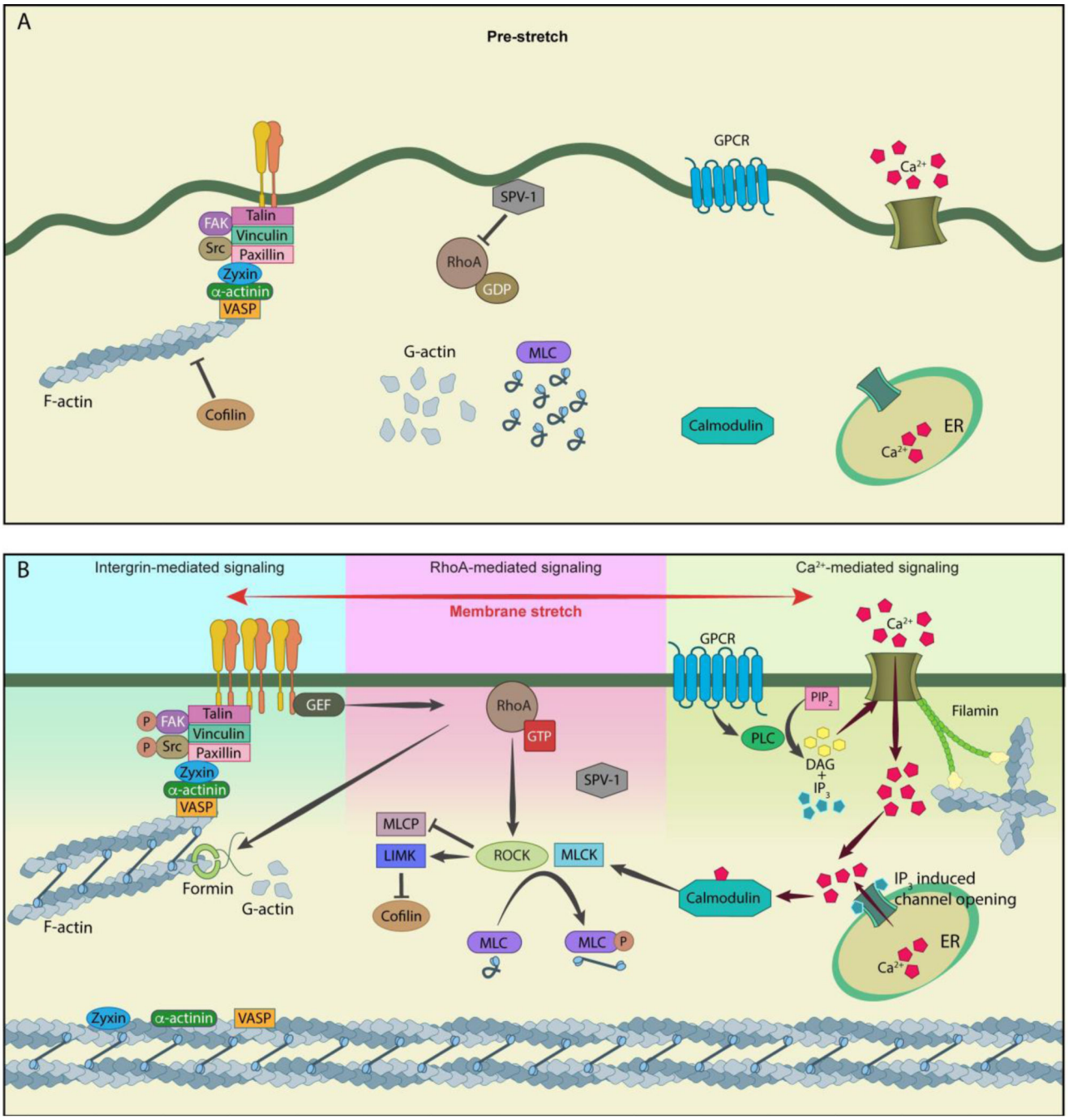

Figure 2. Stretch-induced mechanotransduction pathways leading to actomyosin contractility

(A) Schematic depicting the interiors of a cell in a pre-stretch condition. The RhoGAP SPV-1 is attached to the membrane via its F-BAR domain and actively inhibits RhoA, calcium levels in the cells are low, myosin II is primarily inactive and the level of F-actin is low because of little polymerization and severing by cofilin. (B) Schematic depicting activation of the integrin-mediated pathway, RhoA-mediated pathway and calcium-mediated pathway, in response to stretch. Activation of integrins results in assembly of focal adhesions, Src and FAK phosphorylation, redistribution of zyxin, VASP and alpha-actinin to

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reinforce stress fibers and recruitment of RhoGEFs. Activation of RhoA activates downstream pathways resulting in phosphorylation and activation of myosin. In parallel, actin polymerization is enhanced by RhoA-mediated activation of formins and inhibition of the actin-severing activity of cofilin. Activation of calcium(Ca^{2+})-mediated signaling induces calcium-dependent steps ultimately resulting in myosin activation through MLCK. Filamin functions as a link between calcium influx and actin. All three pathways converge at activation of the actomyosin contractile machinery and consequently increase intracellular tension.