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The actin cytoskeleton in myofibroblast differentiation: Ultrastructure defining form and driving function

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Abstract

Myofibroblasts are modified fibroblasts, characterized by the presence of a well-developed contractile apparatus, and the formation of robust actin stress fibers. These mechanically active cells are thought to orchestrate extracellular matrix remodeling during normal wound healing in response to tissue injury, and in aberrant tissue remodeling found in fibrosing disorders. This review surveys the understanding of the role of actin stress fibers in myofibroblast biology. From its original description as a defining ultrastructural and morphologic feature, to well-accepted observations demonstrating its participation in contraction, focal adhesion maturation, and extracellular matrix reorganization, and finally to more recent observations demonstrating its role in transducing mechanical force into biochemical signals, transcriptional control of genes involved in locomotion, contraction, and matrix reorganization, and the localized regulation of mRNA translation. This breadth of functionality of the actin stress fiber serves to reinforce and amplify its mechanical function, via induced expression of proteins that themselves augment contraction, focal adhesion formation, and matrix remodeling. In composite, the functions of the actin cytoskeleton are most often aligned, allowing for the integration and amplification of signals promoting both myofibroblast differentiation and matrix remodeling during fibrogenesis.

Defining Features of the Myofibroblast

Morphology

In the 1970s, Gabbiani and colleagues [1,2,3] described in detail the presence of a modified fibroblast in the granulation tissue of contracting wounds. By electron microscopy (EM), these fibroblasts were morphologically distinct from “normal” fibroblasts with the presence of large bundles of microfilaments running parallel with the long axis of the cell (whereas normal tissue fibroblast had few or none [1]. These microfilaments often had periodic extensions that connected with external fibronectin fibrils in a parallel orientation termed the fibronexus[4]. The area of the cell membrane where these connections were made was noted to be electron dense (and were later determined to be focal adhesion sites)[5]. These cells lacked the presence of a true basement membrane, instead having a discontinuous electron dense fibrillar structure adjacent to the external cell membrane (later determined to be fibronectin [6]), had extensive rough endoplasmic reticulum which is consistent with fibroblasts and contrasts to differentiated smooth muscle cells, and folded nuclei suggestive of a contractile cell. Due to the presence of: 1) morphologic features reminiscent of EM structures seen in smooth muscle cells, such as densely packed microfilaments and membrane-associated dense-bodies[5,7]; 2) strong immunofluorescent staining with smooth

muscle alpha-actin antiserum [3], and 3) the ability of these cells to contract granulation tissue [1,2,3], these modified fibroblasts displayed a phenotype intermediate between fibroblasts and smooth muscle cells and were termed “myofibroblasts”.

One of the most readily identifiable morphologic features of the myofibroblast in granulation tissue is the presence of prominent cytoplasmic microfilaments[1,2,3]. These cytoskeletal structures are readily recapitulated by growth of fibroblasts on plastic or glass substrates in culture. Amazingly, these observations had been reported in fixed cells dating to 1899 (see [8]), but were discarded as artifacts of the fixation process. In cell culture, these microfilaments are visible by both EM and light microscopy[8], and were determined to consist of polymerized and bundled actin [9]. Due to a lack of similar microfilament bundles in normal dermal fibroblasts[3,10], it has been argued that actin filament formation and the myofibroblast phenotype itself may be an artifact of cell culture conditions, however, subsequent *in vivo* and *ex vivo* investigations have demonstrated the presence of myofibroblasts in normal organs, normal granulation tissue, in tissues responding to localized injury, and in tissue from conditions characterized by an exuberant fibrotic response [11]. These early initial investigations established the importance of the configuration of the actin cytoskeleton to the phenotypic and morphologic definition of the myofibroblast. Forty years of subsequent *in vitro* and *in vivo* investigations would reveal the importance of this structure to a multitude of myofibroblast functions (reviewed below).

Gene Expression Characterizing the Myofibroblast

The concept of the myofibroblast has expanded far beyond its original morphologic definition, with a corresponding increase in understanding of the role the myofibroblast plays during the normal wound healing response and during aberrant tissue remodeling seen in fibrotic disorders. Myofibroblasts participate in the production of matrix proteins and autocrine and paracrine mediators of the fibrotic response, and display enhanced cell survival under these conditions[12,13,14]. Myofibroblast undergoing differentiation exhibit profound changes in their gene expression profile[15] which serves to both amplify the capacity of the fibroblast to serve as a contractile cell and as an effector cell for extracellular matrix reorganization and remodeling.

The gene expression repetoire of the activated myofibroblast is extensive, and a complete catalogue of all modified genes is beyond the scope of this review. However, several key genes have been identified that characterize the myofibroblast phenotype and have an established link to myofibroblast function. While none of these genes are specific to myofibroblasts, they lend insight into the functional capacity and role of this cell in the response to tissue injury. Myofibroblast differentiation is associated with significant upregulation of extracellular matrix components such as fibronectin, including the critical splice variant EDA fibronection[16,17], multiple isoforms of collagen[18,19,20], proteoglycans, such as tenascin C, [21,22]; matrix metalloproteinases (MMPs) and their inhibitors (TIMPs)[23,24], serine proteases such as tissue-type Plasminogen Activator (t-PA) and urokinase-Plasminogen Activator (uPA), Plasminogen-activator inhibitor-1 (PAI-1) [25,26], and proteins involved in cell-matrix coupling, such as integrin and glycoprotein receptors. Additionally, genes that comprise the enhanced contractile machinery of the cell also are profoundly upregulated[27,28]. One protein of particular interest is a component of the actin cytoskeleton, the smooth muscle associated actin isoform, smooth muscle (SM)-α-actin (α-SMA). Although α-SMA was originally thought to be tissue restricted (hence its name), it has also identified as a protein expressed by myofibroblasts during wound healing and fibrosis[11,12,13,29,30,31] and is a component of the actin stress fiber in this context [32]. Historically, α-SMA has been widely utilized as a biochemical marker for the fully differentiated myofibroblast[11,12].

Functions of the Myofibroblast

Myofibroblasts have been found in a wide variety of normal and aberrant responses to tissue injury. Examples of clinical conditions and disease states where myofibroblast biology may play a role include the normal physiologic response to trauma and tissue injury, tissue responses to ischemia/reperfusion and vascular occlusion or thrombosis, and disorders of aberrant wound healing such as pulmonary, renal, liver, and cardiac fibrosis. Myofibroblasts have also been identified *in vivo* in normal, non-injured connective tissue, where they play a role as a contractile cell regulating normal organ function. For example, myofibroblasts have been identified in subpopulations of alveolar interstitial cells, intestinal pericryptal cells, and in hepatic perisinusoidal cells, as well as others[33]. In response to tissue injury, such as occurs from mechanical tissue disruption of the skin (dermal wounds), the myofibroblast serves to produce and remodel granulation tissue, producing matrix components and ultimately facilitating contraction and closure of the wound[3,14,34]. While the model of dermal wound closure has provided the most robust information on myofibroblast function during tissue healing, other forms of tissue injury also reveal participation of myofibroblasts to resolve the “wound”. This includes: the fibroproliferative phase of the clinical syndrome of acute lung injury, characterized by diffuse epithelial injury from infection or toxic insult followed by fibroblast proliferation and remodeling of the injured and inflamed alveolar architecture [35], the remodeling response to the ischemic insult of a myocardial infarction, where healing of the ischemic tissue results in replacement of cardiomyocytes with a fibrotic scar[36], and the local response to vascular thrombosis where infiltration of myofibroblasts into the clot results in its organization and stabilization over time[37]. Myofibroblasts are implicated in the stromal reaction around epithelial tumors, where they may play an important role in remodeling the normal tissue matrix, creating a more permissive environment for the invasion of cancer cells [14,33,38,39]. Finally, myofibroblasts are also found in abundance from pathologic specimens in disease states where the initial tissue injury may be obscure or temporally remote. These disorders are primarily characteristized by exuberant and non-resolving progressive fibrosis. This category of disease includes entities such as idiopathic pulmonary fibrosis[30], interstitial fibrosis of the kidney[40], liver cirrhosis[41], Dupuytren's contracture[42], and hypertrophic scars[43].

While initial tissue injury or disruption is a component of all of these disorders, it is the myofibroblast which acts as central mediators of the architectural disruption and extracellular matrix reorganization that occurs in response. This is due to its role as a site of synthesis and secretion of: 1) matrix metalloproteinases, which may allow for the dissolution of pre-existing matrix architecture; 2) new matrix components, contributing to the remodeling which occurs during the wound healing response; and 3) tissue-inhibitors of both MMPs, providing a balance of matrix acculumation. However, beyond being solely synthetic cells, the contractile function of the myofibroblast allows for active reorganization of the newly synthesized matrix components. This includes facilitating incorporation and remodeling of fibrinogen fibrils into the matrix, and via the transmission of tension to the matrix and its component proteins via isometric contraction.

To illustrate these diverse roles in fibrotic conditions, consider the pathobiology of idiopathic pulmonary fibrosis (IPF), where the defining pathologic lesion is the fibroblastic focus, a collection of linearly arranged myofibroblasts lying on the luminal side of a disrupted basal lamina. This accumulation of myofibroblasts have been identified as sites of synthesis for early matrix elements including pro-collagen I and EDA fibronectin. These features of the fibroblastic focus are similar to the pathology found in dermal wounds. In direct approximation with these pathologic lesions is often a disordered epithelial layer or epithelial sloughing, suggestive of previous epithelial cell injury[30]. This has led to speculation that it is the myofibroblast may contribute the disruption of the basal lamina via localized expression of matrix metalloproteinases [44,45], followed by the secretion of a

provisional matrix high in proteoglycans such as hyaluronan[46] on the luminal side of the original basal lamina. This new matrix "edge" would then serve as a site of further fibroblast migration. While temporal relationships are difficult to dissect on pathologic specimens, presumably fibroblasts would then serve to restructure this provisional matrix, with the expression of early collagen forms, and EDA fibronectin (both of which are found in association with early fibroblastic foci). Simultaneously, inhibitors of proteinases such as TIMPs are expressed which promote matrix accumulation[45]. As the fibroblastic focus continues to develop, myofibroblasts promote further matrix remodeling with the formation of fibrillar fibronection and the production and incorporation of mature collagen forms. Differentiated myofibroblasts demonstrate higher levels of contractile force (due to the myofibroblast contractile apparatus) and this process results in a stiffer, more compact configuration of the lung architecture. Examples such as this suggest that despite effecting disparate organs with distinctive disease tempos, that many components of myofibroblast behavior are preserved.

Although a broad-array of morphologic, biochemical, and functional alterations characterizes the myofibroblast phenotype and its role in the above mentioned disorders, the remainder of this review will focus on the formation of the actin stress fiber, and its role in mediating myofibroblast function.

Composition and formation of the actin stress fiber

A comprehensive review of the composition of the actin stress fiber has been recently published[47], and the reader is referred to this review for detailed discussion. Briefly, actin stress fibers are composed of bundles of polymerized actin filaments[9,48], contiguous with the site of modified focal adhesion complexes [4,6,49] providing a trans-membrane link to components of the extra-cellular matrix. The transmembrane molecules which form the critical linkage between the matrix and actin cytoskeleton at focal adhesion sites are heterodimers of the α and β subunits of integrin molecules [50]. In fibroblasts and myofibroblasts, the heterodimers $\alpha_5\beta_1$ or $\alpha_v\beta_3$ constitute the integrins involved in focal adhesion complex formation at cell contact sites associated with prominent actin stress fibers[49,51,52]. On the external surface of the cell, integrin receptors bind to specific (RGD) sequences found on matrix molecules such fibronectin or vitronectin [50,53], anchoring the cell membrane to the matrix. On the cytoplasmic face, upon integrin attachment and clustering, a focal adhesion complex is rapidly assembled and consists of several proteins in high abundance including talin, vinculin and paxillin, and multiple assessory proteins characterized by both integrin-interacting and actin-interacting domains[50,51,52]. Integrins and many of the associated focal adhesion proteins have actin binding domains, and the cytoplasmic face of a forming focal adhesion complex serves as a nucleating site for actin-filament formation. In concert with the initial formation of actin polymers at sites of focal contact, several proteins closely associated with the cytoplasmic face of the focal contact cross-link individual actin filaments, the most important of which is α-actinin [54,55]. While α-actinin is localized at sites of focal contact, it is also found in a periodic pattern along the length of the actin stress fiber, serving to bundle individual actin filaments together, forming a larger actin "stress fiber". Finally, non-muscle myosin is arranged periodically along the stress fiber[56], alternating with sites of α-actinin binding, and has dual functions: 1) augmentation of actin-filament bundling (via is cross-linking role), and 2) the generation of contractile force on actin filaments via Rho-dependent signaling (For a review of stress fiber contraction, see [47,57,58]).

Interestingly, the particular actin isoform that comprises the actin stress fiber impacts its formation and function. Actin has six isoforms, all of which can participate in the formation of the stress fiber. Four of the isoforms, α-smooth muscle actin (α-SMA), γ-smooth muscle

actin, α-skeletal muscle actin, and α-cardiac actin are tissue restricted, while β-actin and γ-actin are ubiquitously expressed in all cells[59]. Myofibroblasts have cytoplasmic β- and γ-actin isoforms, both of which participate in actin stress fiber formation, with subpopulations of myofibroblasts expressing the α-SMA isoform in the basal state, both *in vivo* and in cell culture. α-SMA expression is strongly induced in myofibroblast populations under conditions of increased isometric tension or under stimulation with Transforming-Growth Factor-β1. When present, α-SMA is rapidly incorporated into actin stress fibers[32,60] and results in an increased capacity for contractile force generation by the myofibroblast[61,62].

The signaling required to form or maintain the actin stress fiber is mediated by members of the Rho family of small GTPases [58,63]. Inhibition of Rho by the *clostridium botulinium* C3 toxin, results in the disassembly of actin stress fibers[64]. Likewise, microinjection of Rho proteins results in the *de novo* assembly of actin stress fibers[65]. While RhoA is the Rho family protein member with the most established role in actin stress fiber formation ectopic expression of either RhoA, RhoB, or RhoC can induce actin stress fiber formation[66]. Of particular note, *de novo* expression of the Rho family member RhoB, is required for actin stress fiber formation during TGF-β-induced myofibroblast differentiation[67]. Rho proteins mediate downstream signaling via their effectors: the serine/threonine kinases ROCK1 and ROCK 2[68], or members of the mammalian diaphanous family of formins, such as mDia1. ROCK1 and ROCK 2 can differentially regulate targets within the cell, with ROCK1 required for assembly of stress fibers, while ROCK 2 is dispensible[69], but may be more important for the myosin II-mediated fibronectin matrix assembly via effects on myosin light chain phosphorylation and subcellular localization[70]. While several members of the formin family exist, mDia1 is implicated in stress fiber formation[71,72,73]. Both ROCK1 and mDia1 are required to produce the characteristic thick actin stress fibers seen with Rho stimulation[72,74,75].

In addition to promoting actin nucleation and filament assembly, Rho-dependent signaling also ultimately results in the phosphorylation of myosin light chain and actomyosin contraction[58,76], for details see [47]. This parallel signaling also is required for and promotes the formation of actin stress fibers, as inhibition of myosin filament assembly, myosin ATPase activity, or myosin light chain kinase activity abrogates actin stress fiber formation[58,77,78,79,80].

Control of actin stress fiber formation by the extracellular matrix

The matrix environment interacting with the fibroblast is also critical for the induction of features consistent with myofibroblast differentiation, such as the formation of actin stress fibers. The observation that fibroblasts in many (but not all) normal tissues did not have the presence of actin microfilaments [1] led to speculation that physical or biochemical characteristics of granulation tissue may influence the morphology of participating fibroblasts. Investigations using deformable collagen gel matrices, designed to mimic *in vivo* conditions, further supported this hypothesis[81,82]. In floating, unattached gels, any tension that created fibroblast contraction is rapidly transmitted to the gel matrix, resulting in contraction of the gel. In this circumstance, cells are not under persistent tension, and actin stress fibers are not observed[81]. In contrast, fibroblasts cultured in collagen matrices which are attached and unable to contract, the rapid appearance of actin stress fibers is seen, consistent with myofibroblast morphology[83]. Subsequent release of these same matrices from attachment results in actin stress fiber disassembly[83,84,85]. Similar results have been observed in other models of variable matrix tension or force application[86,87,88]. Thus, the transmission of tension to the fibroblast appears to be important in inducing the actin cytoskeleton and promoting the myofibroblast phenotype[62,85,89].

Transmission of matrix tension to fibroblasts is mediated, at least in part, via integrins (in particular the β subunits, β_1 and β_3) found in the focal adhesion complex, as blocking antibodies against the β1 subunit results in loss of actin stress fibers and rounding of the cell[89]. With respect to the myofibroblast specifically, the particular molecular composition of the focal adhesion may also be important in mediating force transmission and actin cytoskeletal rearrangement, as large, so called “supermature” focal adhesions are required for the recruitment of α-SMA isoform into the stress fiber[90]. While matrix stiffness can induce changes consistent with the myofibroblast phenotype, it also appears to be a required precondition for full myofibroblast differentiation by Transforming Growth Factor-β1, as treatment of cells growing on floating collagen matrices does not result in the induction of α-SMA or stress fiber formation[89]. Matrix tension alone is insufficient for complete myofibroblast differention, as treatment with TGF-β1 further augments actin stress fiber formation on stiff substrates, along with the expression of biochemical markers characteristic of the fully differentiated myofibroblast, such as α-SMA. These observations have led to the concept of the “protomyofibroblast” and myofibroblast, with matrix stiffness inducing the formation of morphologic alterations, such as partial focal adhesion enlargement and the formation of actin stress fibers, and TGF-β inducing α-SMA, further augmenting actin stress fibers and full maturation of the focal adhesion complex, consistent with a fully differentiated myofibroblast[11].

The molecular composition of the matrix itself can also exert important influence on myofibroblast differentiation. Early studies of transformed fibroblasts showed that addition of soluble fibronectin alone was able to induce actin stress fibers [91]. Additionally, the presence of the EDA splice isoform of fibronectin is required, but not sufficient for myofibroblast differentiation[16].

Stress fiber formation in response to Transforming Growth Factor-β1 (TGF-β1)

TGF-β1 is the most potent and established stimulator of myofibroblast differentiation. TGF-β1 can be release in an autocrine or paracrine fashion, and is incorporated in complex with its latent activating protein and latent binding protein in an inactive state into the extracellular matrix is association with α_v containing integrins[92,93]. Upon either proteolytic cleavage of the TGF-β1 complex or via the application of tension to the associated integrin, TGF-β1 can be released into its active form where it can bind to its receptor on the surface of nearby cells[92,94]. Under stimulation by TGF-β1, fibroblasts respond by altering their ultrastructure, with an increase in cytoskeletal stress fiber formation[95,96], as well as their gene expression profile, with *de novo* expression cytoskeletal and contractile proteins normally found within smooth muscle cells, modified focal adhesion complexes[49,90,97], and components of the extracellular matrix[11,18,19,98,99,100,101]. TGF-β1 has been extensively implicated in the pathogenesis of disorder tissue fibrosis [102,103,104,105,106,107,108].

TGF-β1 canonically signals through its transmembrane-receptor serine/threonine kinases to phosphorylate receptor-associated Smad proteins (Smad2/3), followed by their subsequent heterotrimerization with co-activator Smad4 and nuclear translocation of the complex, which drives the transcription of TGF-β-responsive genes through activation of the Smad-binding elements (SBEs) on their promoters[100,109,110] (Fig. 1A). TGF-β1 can also recruit non-Smad pathways to activate the MAP kinases ERK, p38 and JNK. Activation of ERK by TGF-β occurs through a recruitment of adaptor protein, ShcA by TGF-β receptor, followed by stimulation of Ras-mediated signaling that is common for receptor tyrosine kinases [111] (Fig. 1B). Activation of JNK and p38 MAP kinases by TGF-β occurs via recruitment of TGF-β-activated kinase TAK1 by TRAF6 and TAK1 binding protein (TAB), with TAK1 activating the corresponding upstream kinases (MEK) for JNK and p38 [112,113,114]. In addition, p38 can be also activated through a direct interaction with TAB1

independent on MEKs [115](Fig. 1C). TGF-beta can also activate AKT, initially through a Smad-dependent expression of miR-192 that downregulates a transcriptional repressor, Zeb2, leading to upregulation miR-216a and miR-217. miR-216a and miR-217 downregulate the expression of phosphatase and tensin homolog, PTEN. As a result, PIP3 levels are increased leading to activation of AKT [116,117] (Fig. 1D). Finally, the "non-canonical" signaling of TGF-β also includes activation of Rho small GTPases leading to stress fiber formation [118,119,120,121], although this process may be indirectly dependent on Smad signaling[122] (Fig. 1E).

Activation of Rho is mediated by a large family of guanine exchange factors (RhoGEFs) whose activity is induced by multiple stimuli [123], however, at least in fibroblasts, TGF-β receptor signaling is not directly linked to RhoGEFs. Instead, TGF-β drives the Smad-dependent expression of endocrine and intracellular molecules that, in turn, promote Rho activation. For example, TGF-β1 can promote a Smad-dependent expression of sphingosine kinase-1 (SK-1) producing sphingosine 1-phosphate (S1P), which, in turn, activates G protein coupled receptor signaling to RhoA activation, and α-SMA expression through leukemia-associated RhoGEF (LARG) [124,125,126,127]. TGF-β can also upregulate the expression of GEFs in a Smad-dependent manner, including Net1 and GEF-H1/Lfc, which mediate TGF-β-induced Rho activation [128,129,130]. Together, these studies may suggest that TGF-β-induced Rho activation and stress fiber formation may be mediated by a "non-canonical", but Smad-mediated mechanism.

Roles of the Actin Cytoskeleton in Myofibroblast Function

Focal Adhesion Assembly

The focal adhesion (FA) complex in myofibroblasts serves as an anchoring point to the matrix, providing a stable linkage from matrix to cell, allowing the transmission of intracellular contractile forces to the surrounding matrix, and subsequent tissue contraction. Focal adhesions also serve as a nucleating site for the formation and strengthening of actin stress fibers, and a scaffold for associated signaling molecules[50,52,57]. In this light, focal adhesion formation and maturation is an important process for myofibroblast differentiation.

Focal adhesion formation in motile cells such as fibroblasts is a complex process, initiated by the ligation and clustering of transmembrane integrins and signaling via rhoGTPases, which induce actomyosin contractility and actin stress fiber formation[52,57]. Actomyosin contractility and the generation of intracellular tension are required for focal adhesion formation, as specific inhibitors of myosin contractility can disrupt both actin stress fiber formation and focal adhesion assembly[58,80,131]. Likewise, integrity of the actin cytoskeleton is required for this formation as disruption with cytochalasin or latrunculin A results in the disappearance of focal adhesions[49,87,132]. These results are all consistent with the current model of focal adhesion formation and enlargement that depends on the transmission of tension via the actin cytoskeleton and actomyosin contraction[88].

Expanding upon this concept in models of myofibroblast differentiation, Hinz et al. showed that the incorporation of α-SMA into the actin stress fibers was associated with the enlargement of focal adhesion complexes in myofibroblasts[97], and that blocking antibodies preventing α-SMA incorporation into stress fibers prevented further enlargement of the focal adhesion, resulting in decreased attachment strength. These observations are consistent with the role of the α-SMA isoform in the generation of increased contractile force by the actomyosin contractile apparatus upon α-SMA incorporation into the stress fiber during myofibroblast differentiation. This increased force is presumably transmitted to the focal adhesion site resulting in further tension-dependent recruitment and assembly of focal adhesion associated molecules. While the generation of tension by the actin stress fiber

and its transmission to the focal adhesion site is required for assembly, mechanisms responsible for the recruitment of individual molecules in response to differences in tension have not been fully determined. One potential mechanism is seen in talin-dependent vinculin to the focal adhesion, where tension dependent stretching of the talin molecule exposes the vinculin binding site[133]. Whether this type of mechanism may be more ubiquitous during focal adhesion assembly and maturation is an area of active inquiry[134].

The formation of the focal adhesion complex/actin stress fiber junction in motile cells also correlates with the scaffolding of several signaling molecules at this site. This configuration allows for the transduction of extracellular or intracellular forces into biochemical signals, so called “mechanotransduction”[51,135,136,137]. This “sensing” mechanism is activated by both ligand binding to integrins, such as β1-integrin, and integrin molecule clustering[51], leading to the recruitment of vinculin (by talin)[133], additional focal adhesion associated proteins, and p125 focal adhesion kinase (FAK)[138]. Extensive tyrosine phosphorylation is associated with FA formation[57,139], with targets include multiple FA-related proteins on their SH2 domains[137,140], with downstream effectors including the small GTPases, Rho and Rap1 [141,142,143]. Tyrosine phosphorylation appears to be required for the maintenance and additional incorporation of FA proteins into the FA complex, as inhibition by tyrosine kinase inhibitors results in disassembly of the FA complex[51,57,137]. These signals have myriad downstream targets that affect cell behavior such as proliferation, migration, and cell survival, conferring an important role for the actin stress fiber/focal adhesion complex in the transduction of biochemical signals[144,145].

Extracellular Matrix reorganization

The myofibroblast is an active cell in reorganizing the extracellular matrix (ECM) and its components during wound healing. In particular, much data supports the role of the fibroblast in fibronectin fibril assembly and remodeling[146,147,148]. Fibronectin is a ligand for integrins found on the fibroblast, in particular $\alpha_5\beta_1$[51,149,150]. Upon attachment of fibroblasts to the ECM, integrin binding to fibronection and receptor clustering results in incorporation of soluble fibronectin into the matrix [151], as well as the reorganization of matrix bound fibronectin in to organized fibrils[152]. On the cytoplasmic face of integrin attachment, actin filament formation coincides with the formation of fibronectin fibrils on the extracellular face of focal adhesions[152,153]. Importantly, disruption of the actin cytoskeleton with cytochalasin B results in dispersion of fibronectin fibrils[152,154], demonstrating a requirement for an intact actin cytoskeleton for fibrillogenesis. This finding is explained by the following observations establishing actomyosin-mediated contractility and force generation as a prerequisite for several components of fibronectin fibrillogenesis. To facilitate soluble fibronectin incorporation into the fibrils of the ECM, Rho-dependent tension must be generated to convert fibronectin from a compact conformation to a less compact conformation[155,156,157]. This process exposes molecular modules that allow fibronectin-fibronectin cross-linking of the mature fibril [146,147]. Rho-activation occurs via either integrin-dependent signaling, or via activation by associated transmembrane attachment receptors, such as syndecan 4 or tenascin-C[158,159]. The net result of these signals is the transmission of tension to the fibronectin matrix [155,156], which is required for the assembly of the fibronectin matrix[160]. At specialized attachment sites called fibrillar adhesions comprised of the integrin α5β1 and enriched for the focal adhesion protein tensin, further remodeling of fibronectin fibrils occurs [161,162]. These adhesion structures are mobile with respect to the ECM with actin stress fiber-mediated centripetal dislocation over time[163]. Fibrillar adhesion mediated stretching of fibronectin polymers serves to expose sites along the fibronectin molecule allowing further incorporation and matrix remodeling[146,148,164].

Contraction of the healing wound

A ubiquitous characteristic of granulation tissue healing in adults is the presence of wound contraction in association with granulation tissue maturation. Additionally, granulation tissue can generate contractile force in response to soluble agonists [2]. In intact granulation tissue, the appearance of actin stress fibers correlates with the generation of contractile force [62], and contraction of untethered collagen gels by fibroblasts requires the presence of intact actin microfilaments[85]. Wrinkling of collagen lattices is mediated most strongly by cells with well developed cytoskeletons, such as fibroblasts [165], and disruption of the actin cytoskeleton with agents such as cytochalasin B or cytochalasin D fully inhibits this ability [81,166]. Furthermore, isolated stress fibers have been shown to shorten in response to ATP and Mg^{++}[167,168,169] suggesting an intrinsic ability of actin stress fibers to shorten. Stress fiber contraction is controlled by the Rho-dependent regulation of non-muscle myosin [47,58] which associates with the stress fiber [56]. However, in intact cells stress fibers are tightly associated with focal adhesion complexes[6], resulting in the generation of isometric tension[165] in situations where focal adhesions are tightly bound to an immobile substrate. Further remodeling of the actin microfilament can mediate the generation of additional contractile force, both in cell culture systems[170] and in intact granulation tissue[62]. This system serves to further remodel the granulation tissue matrix elements (such as fibronectin), and facilitate wound closure[11].

Tail retraction during migration

While early investigations suggested that the formation of actin stress fibers may be important for migratory capacity, subsequent investigations showed that the generation of force by actin stress fibers was beyond what is necessary for migratory cells[165]. Cells with a robust actin stress fiber system (contractile phenotype) were observed to be less migratory [171], and that highly motile cells generated the weakest tractional forces[165]. Targeted disruption of actin stress fiber formation converts cells to a more migratory phenotype[172,173].

Despite this, Rho-dependent actomyosin-based contraction are clearly important for tail retraction of highly motile cells[174,175,176,177], with actin stress fibers oriented in the direction of locomotion[178,179]. Thus the localization of stress fiber formation is clearly important for regulation of migratory capacity. For full discussion of the role of actin stress fibers in migration, see [47].

Localization of Translational Machinery

In addition to the well established functional roles of the actin cytoskeleton of the myofibroblast contractile and motile function, emerging evidence implicates the actin cytoskeleton in the orchestration of precise spatiotemporal localization of gene expression[180,181]. This can occur via the regulated intracellular localization of certain mRNA transcripts, such as β-actin mRNA. The so-called “zipcode” hypothesis of mRNA localization, occurs via interaction of specific ribonucleotide sequences within the 3’ UTR with intracellular RNA-binding proteins, allowing for subcellular targeting of specific transcripts to areas where *de novo* gene expression is required[182], such as in the formation of lamellipodia in migrating fibroblasts. This process appears to be important in cellular morphogenesis, and is conserved across species[183]. Subcellular targeting of β-actin mRNA occurs in response to growth factors[184] is required for the formation of actin stress fibers[185] and focal adhesions[186,187] in fibroblasts, and it is tempting to speculate that this process may be operative during myofibroblast differentiation induced by varied mechanisms. Both actin and microtubule filaments have been implicated in the movement of zipcode containing mRNA[188,189,190]. In fibroblasts, actin filaments co-localize with mRNA, often at sites of focal adhesions[186,187], and this co-localization is dependent on

an intact actin cytoskeleton and rho-mediated actomyosin contraction[191] [192,193]. Recruitment of β-actin mRNA to the edge of the fibroblast can be induced via the application of integrin-dependent tension or via stimulation with serum[184,193,194]. Additionally, components of the translational apparatus itself, such as polyribosomes[187,195,196,197,198,199] and elongation-initiation factor 1a (eIF1a) [200,201,202,203,204], localize to the actin cytoskeleton, and require actin filament integrity to maintain this localization [205]. Zipcode binding protein-1 (ZBP-1) was the first protein identified in eukaryotic cells to bind to mRNA in the 3'-UTR and mediate spatial localization via its interaction with actin. ZBP-1 also acts as a translational repressor via inhibition of 80S ribosome formation. Phosphorylation of ZBP-1 by SRC near the cell edge results in the release of the inhibition of β-actin mRNA translation by ZBP1[180]. Thus, localized translation of mRNAs containing zipcode sequences requires coordinated movement of proteins comprising the polyribosome, translational machinery, and the zipcode containing mRNA via actin filamentsto subcellular locations for assembly. Once at this site, release of translational inhibition is mediated via SRC-mediated phosphoryation of ZBP-1, allowing localized gene expression to occur[180].

One potential functional consequence of regulated targeting of polysomes and associated mRNAs is localized translation of cytoskeletal proteins important for cell polarization or focal adhesion formation during cell functions such as migration [206]. This is supported by direct visualization of zipcode-contating mRNA encoding components of the actin cytoskeleton undergoing translation at sites of focal contacts[207,208]. Furthermore, disruption of mRNA targeting using antisense oligonucleotides targeted to the zipcode region of β-actin mRNA resulted in the inhibition of the directionality of lamellipodia formation during migration[209]. Additionally, homeostatic mRNA targeting is also important in the development of normal cell polarity and in inhibiting metastatic potential[210,211,212].

Transcriptional Regulation

Complementing the role of the actin cytoskeleton in translational control of cytoskeletal gene expression, the actin cytoskeleton actively participates in the regulation of transcriptional activity for genes encoding cytoskeletal and contractile elements.

Actin-dependent transcriptional regulation is accomplished via subcellular localization of actin-binding proteins that can serve as coactivators of transcription factors. The best example of this type of regulation is in the control of the activity of the transcription factor, serum response factor (SRF). SRF is a transcription factor that is controls myogenic gene expression during development and smooth muscle cell differentiation[213]. SRF activation in response to serum was originally shown to be dependent on the activity of the small GTPase Rho, which also reorganizes the actin cytoskeleton[214]. Subsequently, actin dynamics were found to be required for SRF activity as well, with induction of actin polymerization leading to induction of SRF-dependent gene expression[215]. This effect appeared to be dependent on levels of the G (monomeric) actin pool. Ultimately, it was determined that this effect was mediated by an actin-binding protein, MKL1/myocardin-related transcription factor-A (MRTF-A), which is known to associate with monomeric (G) actin[216]. Upon polymerization of actin (F-Actin formation), MRTF-A is released from monomeric actin and translocates to the nucleus, associating with SRF to activate SRF target genes.

This signaling mechanism provides an elegant means by which extracellular signals can be transduced into effects on gene expression. This has been established in fibroblasts for hormone receptor-ligand interactions, such as LPA, sphingosine-1 phosphate, and endothelin-1, which are known to act via G protein-coupled receptor activation of Rho

proteins[217,218,219]. Additional evidence supports the role of this mechanism in adhesion receptor-ligand interactions, such as integrin-mediated activation of Rho, during force transduction[220]. Additionally, non-canonical signaling by TGF-β itself appears to activate rho and MRTF-A translocation, upregulate SRF expression and activation during myofibroblast differentiation [221,222,223]. This pathway is also required for epithelial-mesenchymal transition induced by cell-contact disruption or TGF-β during myofibroblast differention[224,225]. Interestingly, TGF-β induces components of the rho/actin/MRTF-A/SRF signaling pathway, such as G-proteins coupled receptor agonists, rho proteins, and rhoGEFs in a smad-dependent fashion. In this way, MRTF-A/SRF activation may be complimentary to smad-dependent gene expression during myofibroblast differentiation, further augmenting the expression of a subset of SRF-dependent genes, beyond levels induced by smads alone, and potentially priming this pathway for additional activation by other agonists (such as GPCR-receptor agonists). This data supports the model of this signaling pathway as an important convergence point for varied cell environment-dependent signals important in promoting myofibroblast differentiation. The actin/MRTF-A/SRF signaling pathway has been demonstrated to controls numerous genes involved in the contractile apparatus, focal adhesion complex, and cell cytoskeleton in various cell systems[226,227]. Given the importance of a cytoskeletal integrity, contractility, and tension on promoting myofibroblast differentiation, SRF-dependent gene expression may serve as a crucial feed-forward mechanism promoting this transition during wound healing and fibrogenesis.

Finally, nuclear actin polymers can also interact with RNA polymerases I, II, III [228,229,230], and this interaction is required for transcriptional initiation. However, it is not clear whether polymerized actin or actinomyosin contractility is required for this function, or whether the configuration of cytosolic actin can contribute to the regulation of this function [230,231]. Future investigations on the role of nuclear actin in the control of transcriptional machinery, as well as the localization of transcription factors and there co-activators will most likely yield new insights into the function of actin in this subcellular compartment.

How might the actin cytoskeleton participate in the integration of these multiple functions of the myofibroblast during the response to injury?

Given the multiple signals impacting the actin cytoskeleton in myofibroblast biology, the temporal dynamics of myofibroblast behavior during wound healing remains difficult to dissect. Despite this several themes are evident, including the presence of mutually reinforcing signaling promoting additional myofibroblast activation and matrix remodeling. A schematic of the interconnected functions impacted by the actin cytoskeleton is shown in Figure 2 and lend some insight into mechanisms of myofibroblast biology. Upon fibroblast migration to injured tissue under the influence of chemoattractants, the fibroblast will likely encounter low-level stiffness similar to normal tissue[232]. The fibroblast may express and release matrix metalloproteinases to aid in dissolution of the pre-existing matrix, while at the same time synthesizing and laying down glycoproteins, early collagen forms, and EDA fibronectin, which are dependent on canonical TGF-β/smad signaling, in contrast to TGF-β-dependent rho/actin/MRTF-A/SRF signaling. Simultaneously, upon migrating to a wound site, fibroblasts begin to form more stable focal adhesions, with the corresponding induction and requirement rho signaling/actin stress fiber formation and development of contractile force. Due to this increase in cell contractility, this process is rapidly self-reinforcing with a further induction and enlargement of focal adhesion size[97] and transmission of tension to the surrounding matrix via its linkage to fibronectin. Tension dependent unfolding of fibronectin leads to additional fibronectin cross-linking and incorporation. Intracellularly, the actin cytoskeleton plays a key role in transducing these mechanical signals to

biochemical signals, with FAK-dependent activation of pro-survival signals and the activation of SRF-dependent gene expression via MRTF-A translocation. As the remodeling process progresses, SRF-dependent gene expression of cytoskeletal and contractile genes could contribute to the reinforcement and remodeling of the contractile machinery via the induction of smooth muscle-specific myosin and expression of α-SMA. Overall these events would further augment contractility and the development of intracellular and matrix tension. Complimenting the production of matrix components, the expression of TIMPs could inhibit the dissolution of newly secreted matrix elements, and the myofibroblast would actively incorporate and reorganize the elements, including fibrillar fibronectin. The overall effect of increased contractility and matrix accumulation, incorporation, and reorganization is an increase in the stiffness of the matrix, which provides a positive feedback to intracellular, actin-dependent signaling.

Given the potential role of actin-dependent signaling in integrating multiple inputs promoting myofibroblast differentiation and matrix remodeling, it is an attractive target for pharmacologic intervention in fibrotic disease. Potential points for intervention include the inhibition of Rho-signaling, including ROCK1 or ROCK2, or newly discovered small molecular inhibitors of formins[233]. These approaches would have the benefit of broadly disrupting actin-dependent signaling, but many of these approaches may have significant detrimental effects on normal organ function. A potentially more useful approach may be to attempt to target the adaptive responses that signal via actin during myofibroblast differentiation, thereby disrupting the feed-forward signaling which occurs. One potential strategy is the targeting of actin-dependent transcriptional regulation, via interruption of SRF co-activators. Notably, germ-line deletion of the SRF co-activator MRTF-A yields viable animals that are protected against cardiac fibrosis after myocardial infarction[221].

Summary and Future Directions

The actin myofilament was identified as one of the original phenotypic modulations characterizing the myofibroblast phenotype. Early speculations that this prominent cytoskeletal structure contributed the mechanical functions of the myofibroblast during wound healing and fibrosis were confirmed by experimental data demonstrating its role in migration, contraction, and matrix remodeling. More recent investigations have established additional roles for the actin cytoskeleton in the transduction of mechanical stimuli into biochemical signaling, and transcriptional and translational regulation. In mediating these effects, the actin cytoskeleton serves as a central integrator of divergent signaling inputs including matrix components, soluble growth factors, and physical force inputs. In response to these varied signaling inputs, the actin cytoskeleton can modulate intracellular and extracellular tension generation, focal adhesion formation and matrix remodeling, as well cell signals leading to increased cytoskeletal, contractile, focal adhesion, and matrix gene expression, amplifying these myofibroblast functions during wound healing. In light of these diverse roles, the actin-associated molecules mediating these responses serve as an attractive target for the modulation of myofibroblast behavior during fibrogenesis.

Future research should reveal how the actin cytoskeleton leads to activation of biochemical signals near the focal adhesion complex, either via its role as a scaffold bringing signaling components into close approximation, or perhaps via additional mechanisms of tension-dependent recruitment leading to physical modifications of signaling molecules resulting in their activation. Additional investigations are needed to understand how adaptive responses in modified cells such as the myofibroblast differ from traditional cell models in regard to focal adhesion complex assembly and its effect on matrix interactions. Determining differences such as this may inform how one can best interfere with actin-dependent signaling to inhibit myofibroblast response, without interfering with tissue homeostasis. One

potential set of targets in this response is actin/MRTF-A/SRF-dependent gene expression, which is known to modulate adaptive contractile gene expression, but may also be important in inducing a broader set of adhesion and matrix-associated genes during myofibroblast differentiation. The identification of key components of this response may provide new targets for intervention in a broad set of fibrotic conditions.

In summary, since its original identification as a critical ultrastructural component of the myofibroblast, the actin cytoskeleton has been shown to be involved in a multitude of roles critical to its function. Future studies should clarify these functions and may yield helpful insights into the pathobiology of aberrant fibrotic responses.

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References

1. Gabbiani G, Ryan GB, Majne G. Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. Experientia. 1971; 27:549–550. [PubMed: 5132594]
2. Majno G, Gabbiani G, Hirschel BJ, Ryan GB, Statkov PR. Contraction of granulation tissue in vitro: similarity to smooth muscle. Science. 1971; 173:548–550. [PubMed: 4327529]
3. Ryan GB, Cliff WJ, Gabbiani G, Irle C, Montandon D, et al. Myofibroblasts in human granulation tissue. Hum Pathol. 1974; 5:55–67. [PubMed: 4588257]
4. Singer II. The fibronexus: a transmembrane association of fibronectin-containing fibers and bundles of 5 nm microfilaments in hamster and human fibroblasts. Cell. 1979; 16:675–685. [PubMed: 222466]
5. Eyden B. The myofibroblast: an assessment of controversial issues and a definition useful in diagnosis and research. Ultrastruct Pathol. 2001; 25:39–50. [PubMed: 11297318]
6. Singer II, Kawka DW, Kazazis DM, Clark RA. In vivo co-distribution of fibronectin and actin fibers in granulation tissue: immunofluorescence and electron microscope studies of the fibronexus at the myofibroblast surface. J Cell Biol. 1984; 98:2091–2106. [PubMed: 6373789]
7. Panner BJ, Honig CR. Filament ultrastructure and organization in vertebrate smooth muscle. Contraction hypothesis based on localization of actin and myosin. J Cell Biol. 1967; 35:303–321. [PubMed: 4169225]
8. Buckley IK, Porter KR. Cytoplasmic fibrils in living cultured cells. A light and electron microscope study. Protoplasma. 1967; 64:349–380. [PubMed: 4866690]
9. Goldman RD, Lazarides E, Pollack R, Weber K. The distribution of actin in non-muscle cells. The use of actin antibody in the localization of actin within the microfilament bundles of mouse 3T3 cells. Exp Cell Res. 1975; 90:333–344. [PubMed: 1089542]
10. Ehrlich HP, Hembry RM. A comparative study of fibroblasts in healing freeze and burn injuries in rats. Am J Pathol. 1984; 117:218–224. [PubMed: 6496654]
11. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol. 2002; 3:349–363. [PubMed: 11988769]
12. Desmouliere A, Chaponnier C, Gabbiani G. Tissue repair, contraction, and the myofibroblast. Wound Repair Regen. 2005; 13:7–12. [PubMed: 15659031]
13. Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, et al. The myofibroblast: one function, multiple origins. Am J Pathol. 2007; 170:1807–1816. [PubMed: 17525249]
14. Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saada JI, et al. Myofibroblasts. I. Paracrine cells important in health and disease. Am J Physiol. 1999; 277:C1–C9. [PubMed: 10409103]

15. Kapoun AM, Gaspar NJ, Wang Y, Damm D, Liu YW, et al. Transforming growth factor-beta receptor type 1 (TGFbetaRI) kinase activity but not p38 activation is required for TGFbetaRI-induced myofibroblast differentiation and profibrotic gene expression. Mol Pharmacol. 2006; 70:518–531. [PubMed: 16707625]

16. Serini G, Bochaton-Piallat ML, Ropraz P, Geinoz A, Borsi L, et al. The fibronectin domain ED-A is crucial for myofibroblastic phenotype induction by transforming growth factor-beta1. J Cell Biol. 1998; 142:873–881. [PubMed: 9700173]

17. Borsi L, Castellani P, Risso AM, Leprini A, Zardi L. Transforming growth factor-beta regulates the splicing pattern of fibronectin messenger RNA precursor. FEBS Lett. 1990; 261:175–178. [PubMed: 2307231]

18. Roberts AB, Sporn MB, Assoian RK, Smith JM, Roche NS, et al. Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. Proc Natl Acad Sci U S A. 1986; 83:4167–4171. [PubMed: 2424019]

19. Roberts CJ, Birkenmeier TM, McQuillan JJ, Akiyama SK, Yamada SS, et al. Transforming growth factor beta stimulates the expression of fibronectin and of both subunits of the human fibronectin receptor by cultured human lung fibroblasts. J Biol Chem. 1988; 263:4586–4592. [PubMed: 2965146]

20. Fine A, Goldstein RH. The effect of transforming growth factor-beta on cell proliferation and collagen formation by lung fibroblasts. J Biol Chem. 1987; 262:3897–3902. [PubMed: 3493244]

21. Hanamura N, Yoshida T, Matsumoto E, Kawarada Y, Sakakura T. Expression of fibronectin and tenascin-C mRNA by myofibroblasts, vascular cells and epithelial cells in human colon adenomas and carcinomas. Int J Cancer. 1997; 73:10–15. [PubMed: 9334802]

22. De Wever O, Nguyen QD, Van Hoorde L, Bracke M, Bruyneel E, et al. Tenascin-C and SF/HGF produced by myofibroblasts in vitro provide convergent pro-invasive signals to human colon cancer cells through RhoA and Rac. FASEB J. 2004; 18:1016–1018. [PubMed: 15059978]

23. Overall CM, Wrana JL, Sodek J. Transforming growth factor-beta regulation of collagenase, 72 kDa-progelatinase, TIMP and PAI-1 expression in rat bone cell populations and human fibroblasts. Connect Tissue Res. 1989; 20:289–294. [PubMed: 2558843]

24. Overall CM, Wrana JL, Sodek J. Independent regulation of collagenase, 72-kDa progelatinase, and metalloendoproteinase inhibitor expression in human fibroblasts by transforming growth factor-beta. J Biol Chem. 1989; 264:1860–1869. [PubMed: 2536374]

25. Eddy AA. Molecular insights into renal interstitial fibrosis. J Am Soc Nephrol. 1996; 7:2495–2508. [PubMed: 8989727]

26. Eddy AA. Expression of genes that promote renal interstitial fibrosis in rats with proteinuria. Kidney Int Suppl. 1996; 54:S49–S54. [PubMed: 8731195]

27. Chambers RC, Leoni P, Kaminski N, Laurent GJ, Heller RA. Global expression profiling of fibroblast responses to transforming growth factor-beta1 reveals the induction of inhibitor of differentiation-1 and provides evidence of smooth muscle cell phenotypic switching. Am J Pathol. 2003; 162:533–546. [PubMed: 12547711]

28. Malmstrom J, Lindberg H, Lindberg C, Bratt C, Wieslander E, et al. Transforming growth factor-beta 1 specifically induce proteins involved in the myofibroblast contractile apparatus. Mol Cell Proteomics. 2004; 3:466–477. [PubMed: 14766930]

29. Evans JN, Kelley J, Krill J, Low RB, Adler KB. The myofibroblast in pulmonary fibrosis. Chest. 1983; 83:97S–98S. [PubMed: 6839861]

30. Kuhn C, McDonald JA. The roles of the myofibroblast in idiopathic pulmonary fibrosis. Ultrastructural and immunohistochemical features of sites of active extracellular matrix synthesis. Am J Pathol. 1991; 138:1257–1265. [PubMed: 2024710]

31. Phan SH. Role of the myofibroblast in pulmonary fibrosis. Kidney Int Suppl. 1996; 54:S46–S48. [PubMed: 8731194]

32. Darby I, Skalli O, Gabbiani G. Alpha-smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. Lab Invest. 1990; 63:21–29. [PubMed: 2197503]

33. Desmouliere A, Gabbiani G. Modulation of fibroblastic cytoskeletal features during pathological situations: the role of extracellular matrix and cytokines. Cell Motil Cytoskeleton. 1994; 29:195–203. [PubMed: 7895283]
34. Welch MP, Odland GF, Clark RA. Temporal relationships of F-actin bundle formation, collagen and fibronectin matrix assembly, and fibronectin receptor expression to wound contraction. J Cell Biol. 1990; 110:133–145. [PubMed: 2136860]
35. Pache JC, Christakos PG, Gannon DE, Mitchell JJ, Low RB, et al. Myofibroblasts in diffuse alveolar damage of the lung. Mod Pathol. 1998; 11:1064–1070. [PubMed: 9831203]
36. Porter KE, Turner NA. Cardiac fibroblasts: at the heart of myocardial remodeling. Pharmacol Ther. 2009; 123:255–278. [PubMed: 19460403]
37. Feigl W, Susani M, Ulrich W, Matejka M, Losert U, et al. Organisation of experimental thrombosis by blood cells. Evidence of the transformation of mononuclear cells into myofibroblasts and endothelial cells. Virchows Arch A Pathol Anat Histopathol. 1985; 406:133–148. [PubMed: 3159148]
38. Kunz-Schughart LA, Knuechel R. Tumor-associated fibroblasts (part I): Active stromal participants in tumor development and progression? Histol Histopathol. 2002; 17:599–621. [PubMed: 11962761]
39. Kunz-Schughart LA, Knuechel R. Tumor-associated fibroblasts (part II): Functional impact on tumor tissue. Histol Histopathol. 2002; 17:623–637. [PubMed: 11962762]
40. Meran S, Steadman R. Fibroblasts and myofibroblasts in renal fibrosis. Int J Exp Pathol. 2011
41. Rudolph R, McClure WJ, Woodward M. Contractile fibroblasts in chronic alcoholic cirrhosis. Gastroenterology. 1979; 76:704–709. [PubMed: 421998]
42. VandeBerg JS, Rudolph R, Gelberman R, Woodward MR. Ultrastructural relationship of skin to nodule and cord in Dupuytren's contracture. Plast Reconstr Surg. 1982; 69:835–844. [PubMed: 7071229]
43. Eddy RJ, Petro JA, Tomasek JJ. Evidence for the nonmuscle nature of the Ő̃myofibroblast" of granulation tissue and hypertropic scar. An immunofluorescence study. Am J Pathol. 1988; 130:252–260. [PubMed: 3277440]
44. Hayashi T, Stetler-Stevenson WG, Fleming MV, Fishback N, Koss MN, et al. Immunohistochemical study of metalloproteinases and their tissue inhibitors in the lungs of patients with diffuse alveolar damage and idiopathic pulmonary fibrosis. Am J Pathol. 1996; 149:1241–1256. [PubMed: 8863673]
45. Selman M, Ruiz V, Cabrera S, Segura L, Ramirez R, et al. TIMP-1, −2, −3, and −4 in idiopathic pulmonary fibrosis. A prevailing nondegradative lung microenvironment? Am J Physiol Lung Cell Mol Physiol. 2000; 279:L562–L574.
46. Bensadoun ES, Burke AK, Hogg JC, Roberts CR. Proteoglycan deposition in pulmonary fibrosis. Am J Respir Crit Care Med. 1996; 154:1819–1828. [PubMed: 8970376]
47. Pellegrin S, Mellor H. Actin stress fibres. J Cell Sci. 2007; 120:3491–3499. [PubMed: 17928305]
48. Kreis TE, Winterhalter KH, Birchmeier W. In vivo distribution and turnover of fluorescently labeled actin microinjected into human fibroblasts. Proc Natl Acad Sci U S A. 1979; 76:3814–3818. [PubMed: 291042]
49. Dugina V, Fontao L, Chaponnier C, Vasiliev J, Gabbiani G. Focal adhesion features during myofibroblastic differentiation are controlled by intracellular and extracellular factors. J Cell Sci. 2001; 114:3285–3296. [PubMed: 11591817]
50. Hynes RO. Integrins: bidirectional, allosteric signaling machines. Cell. 2002; 110:673–687. [PubMed: 12297042]
51. Geiger B, Bershadsky A, Pankov R, Yamada KM. Transmembrane crosstalk between the extracellular matrix--cytoskeleton crosstalk. Nat Rev Mol Cell Biol. 2001; 2:793–805. [PubMed: 11715046]
52. Dubash AD, Menold MM, Samson T, Boulter E, Garcia-Mata R, et al. Chapter 1. Focal adhesions: new angles on an old structure. Int Rev Cell Mol Biol. 2009; 277:1–65. [PubMed: 19766966]
53. Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion. Cell. 1992; 69:11–25. [PubMed: 1555235]

54. Lazarides E. Immunofluorescence studies on the structure of actin filaments in tissue culture cells. J Histochem Cytochem. 1975; 23:507–528. [PubMed: 1095651]
55. Lazarides E, Burridge K. Alpha-actinin: immunofluorescent localization of a muscle structural protein in nonmuscle cells. Cell. 1975; 6:289–298. [PubMed: 802682]
56. Weber K, Groeschel-Stewart U. Antibody to myosin: the specific visualization of myosin-containing filaments in nonmuscle cells. Proc Natl Acad Sci U S A. 1974; 71:4561–4564. [PubMed: 4612524]
57. Burridge K, Chrzanowska-Wodnicka M. Focal adhesions, contractility, and signaling. Annu Rev Cell Dev Biol. 1996; 12:463–518. [PubMed: 8970735]
58. Chrzanowska-Wodnicka M, Burridge K. Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. J Cell Biol. 1996; 133:1403–1415. [PubMed: 8682874]
59. Chaponnier C, Gabbiani G. Pathological situations characterized by altered actin isoform expression. J Pathol. 2004; 204:386–395. [PubMed: 15495226]
60. Serini G, Gabbiani G. Mechanisms of myofibroblast activity and phenotypic modulation. Exp Cell Res. 1999; 250:273–283. [PubMed: 10413583]
61. Hinz B, Gabbiani G, Chaponnier C. The NH2-terminal peptide of alpha-smooth muscle actin inhibits force generation by the myofibroblast in vitro and in vivo. J Cell Biol. 2002; 157:657–663. [PubMed: 11994316]
62. Hinz B, Mastrangelo D, Iselin CE, Chaponnier C, Gabbiani G. Mechanical tension controls granulation tissue contractile activity and myofibroblast differentiation. Am J Pathol. 2001; 159:1009–1020. [PubMed: 11549593]
63. Ridley AJ, Hall A. Distinct patterns of actin organization regulated by the small GTP-binding proteins Rac and Rho. Cold Spring Harb Symp Quant Biol. 1992; 57:661–671. [PubMed: 1339704]
64. Chardin P, Boquet P, Madaule P, Popoff MR, Rubin EJ, et al. The mammalian G protein rhoC is ADP-ribosylated by Clostridium botulinum exoenzyme C3 and affects actin microfilaments in Vero cells. Embo J. 1989; 8:1087–1092. [PubMed: 2501082]
65. Paterson HF, Self AJ, Garrett MD, Just I, Aktories K, et al. Microinjection of recombinant p21rho induces rapid changes in cell morphology. J Cell Biol. 1990; 111:1001–1007. [PubMed: 2118140]
66. Giry M, Popoff MR, von Eichel-Streiber C, Boquet P. Transient expression of RhoA, -B, and -C GTPases in HeLa cells potentiates resistance to Clostridium difficile toxins A and B but not to Clostridium sordellii lethal toxin. Infect Immun. 1995; 63:4063–4071. [PubMed: 7558320]
67. Vardouli L, Vasilaki E, Papadimitriou E, Kardassis D, Stournaras C. A novel mechanism of TGFbeta-induced actin reorganization mediated by Smad proteins and Rho GTPases. Febs J. 2008; 275:4074–4087. [PubMed: 18631173]
68. Riento K, Ridley AJ. Rocks: multifunctional kinases in cell behaviour. Nat Rev Mol Cell Biol. 2003; 4:446–456. [PubMed: 12778124]
69. Yoneda A, Multhaupt HA, Couchman JR. The Rho kinases I and II regulate different aspects of myosin II activity. J Cell Biol. 2005; 170:443–453. [PubMed: 16043513]
70. Yoneda A, Ushakov D, Multhaupt HA, Couchman JR. Fibronectin matrix assembly requires distinct contributions from Rho kinases I and -II. Mol Biol Cell. 2007; 18:66–75. [PubMed: 17065553]
71. Hotulainen P, Lappalainen P. Stress fibers are generated by two distinct actin assembly mechanisms in motile cells. J Cell Biol. 2006; 173:383–394. [PubMed: 16651381]
72. Watanabe N, Kato T, Fujita A, Ishizaki T, Narumiya S. Cooperation between mDia1 and ROCK in Rho-induced actin reorganization. Nat Cell Biol. 1999; 1:136–143. [PubMed: 10559899]
73. Zigmond SH. Formin-induced nucleation of actin filaments. Curr Opin Cell Biol. 2004; 16:99–105. [PubMed: 15037312]
74. Leung T, Chen XQ, Manser E, Lim L. The p160 RhoA-binding kinase ROK alpha is a member of a kinase family and is involved in the reorganization of the cytoskeleton. Mol Cell Biol. 1996; 16:5313–5327. [PubMed: 8816443]
75. Watanabe N, Madaule P, Reid T, Ishizaki T, Watanabe G, et al. p140mDia, a mammalian homolog of Drosophila diaphanous, is a target protein for Rho small GTPase and is a ligand for profilin. Embo J. 1997; 16:3044–3056. [PubMed: 9214622]

76. Katoh K, Kano Y, Amano M, Onishi H, Kaibuchi K, et al. Rho-kinase--mediated contraction of isolated stress fibers. J Cell Biol. 2001; 153:569–584. [PubMed: 11331307]
77. Honer B, Jockusch BM. Stress fiber dynamics as probed by antibodies against myosin. Eur J Cell Biol. 1988; 47:14–21. [PubMed: 3068056]
78. Lamb NJ, Fernandez A, Conti MA, Adelstein R, Glass DB, et al. Regulation of actin microfilament integrity in living nonmuscle cells by the cAMP-dependent protein kinase and the myosin light chain kinase. J Cell Biol. 1988; 106:1955–1971. [PubMed: 3290222]
79. Fernandez A, Brautigan DL, Mumby M, Lamb NJ. Protein phosphatase type-1, not type-2A, modulates actin microfilament integrity and myosin light chain phosphorylation in living nonmuscle cells. J Cell Biol. 1990; 111:103–112. [PubMed: 2164027]
80. Volberg T, Geiger B, Citi S, Bershadsky AD. Effect of protein kinase inhibitor H-7 on the contractility, integrity, and membrane anchorage of the microfilament system. Cell Motil Cytoskeleton. 1994; 29:321–338. [PubMed: 7859295]
81. Bell E, Ivarsson B, Merrill C. Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. Proc Natl Acad Sci U S A. 1979; 76:1274–1278. [PubMed: 286310]
82. Bellows CG, Melcher AH, Aubin JE. Contraction and organization of collagen gels by cells cultured from periodontal ligament, gingiva and bone suggest functional differences between cell types. J Cell Sci. 1981; 50:299–314. [PubMed: 6798046]
83. Farsi JM, Aubin JE. Microfilament rearrangements during fibroblast-induced contraction of three-dimensional hydrated collagen gels. Cell Motil. 1984; 4:29–40. [PubMed: 6539173]
84. Mochitate K, Pawelek P, Grinnell F. Stress relaxation of contracted collagen gels: disruption of actin filament bundles, release of cell surface fibronectin, and down-regulation of DNA and protein synthesis. Exp Cell Res. 1991; 193:198–207. [PubMed: 1995294]
85. Tomasek JJ, Haaksma CJ, Eddy RJ, Vaughan MB. Fibroblast contraction occurs on release of tension in attached collagen lattices: dependency on an organized actin cytoskeleton and serum. Anat Rec. 1992; 232:359–368. [PubMed: 1543260]
86. Choquet D, Felsenfeld DP, Sheetz MP. Extracellular matrix rigidity causes strengthening of integrin-cytoskeleton linkages. Cell. 1997; 88:39–48. [PubMed: 9019403]
87. Riveline D, Zamir E, Balaban NQ, Schwarz US, Ishizaki T, et al. Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism. J Cell Biol. 2001; 153:1175–1186. [PubMed: 11402062]
88. Galbraith CG, Yamada KM, Sheetz MP. The relationship between force and focal complex development. J Cell Biol. 2002; 159:695–705. [PubMed: 12446745]
89. Arora PD, Narani N, McCulloch CA. The compliance of collagen gels regulates transforming growth factor-beta induction of alpha-smooth muscle actin in fibroblasts. Am J Pathol. 1999; 154:871–882. [PubMed: 10079265]
90. Hinz B. Masters and servants of the force: the role of matrix adhesions in myofibroblast force perception and transmission. Eur J Cell Biol. 2006; 85:175–181. [PubMed: 16546559]
91. Yamada KM, Yamada SS, Pastan I. Cell surface protein partially restores morphology, adhesiveness, and contact inhibition of movement to transformed fibroblasts. Proc Natl Acad Sci U S A. 1976; 73:1217–1221. [PubMed: 177979]
92. Wipff PJ, Hinz B. Integrins and the activation of latent transforming growth factor beta1 - an intimate relationship. Eur J Cell Biol. 2008; 87:601–615. [PubMed: 18342983]
93. Margadant C, Sonnenberg A. Integrin-TGF-beta crosstalk in fibrosis, cancer and wound healing. EMBO Rep. 2010; 11:97–105. [PubMed: 20075988]
94. Wipff PJ, Rifkin DB, Meister JJ, Hinz B. Myofibroblast contraction activates latent TGF-beta1 from the extracellular matrix. J Cell Biol. 2007; 179:1311–1323. [PubMed: 18086923]
95. Desmouliere A, Geinoz A, Gabbiani F, Gabbiani G. Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. J Cell Biol. 1993; 122:103–111. [PubMed: 8314838]

96. Vaughan MB, Howard EW, Tomasek JJ. Transforming growth factor-beta1 promotes the morphological and functional differentiation of the myofibroblast. Exp Cell Res. 2000; 257:180–189. [PubMed: 10854066]

97. Hinz B, Dugina V, Ballestrem C, Wehrle-Haller B, Chaponnier C. Alpha-smooth muscle actin is crucial for focal adhesion maturation in myofibroblasts. Mol Biol Cell. 2003; 14:2508–2519. [PubMed: 12808047]

98. Ignotz RA, Heino J, Massague J. Regulation of cell adhesion receptors by transforming growth factor-beta. Regulation of vitronectin receptor and LFA-1. J Biol Chem. 1989; 264:389–392. [PubMed: 2462560]

99. Ignotz RA, Massague J. Transforming growth factor-beta stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. J Biol Chem. 1986; 261:4337–4345. [PubMed: 3456347]

100. Leask A, Abraham DJ. TGF-beta signaling and the fibrotic response. Faseb J. 2004; 18:816–827. [PubMed: 15117886]

101. Phan SH. Fibroblast phenotypes in pulmonary fibrosis. Am J Respir Cell Mol Biol. 2003; 29:S87–S92. [PubMed: 14503563]

102. Broekelmann TJ, Limper AH, Colby TV, McDonald JA. Transforming growth factor beta 1 is present at sites of extracellular matrix gene expression in human pulmonary fibrosis. Proc Natl Acad Sci U S A. 1991; 88:6642–6646. [PubMed: 1862087]

103. Santana A, Saxena B, Noble NA, Gold LI, Marshall BC. Increased expression of transforming growth factor beta isoforms (beta 1, beta 2, beta 3) in bleomycin-induced pulmonary fibrosis. Am J Respir Cell Mol Biol. 1995; 13:34–44. [PubMed: 7541221]

104. Phan SH, Kunkel SL. Lung cytokine production in bleomycin-induced pulmonary fibrosis. Exp Lung Res. 1992; 18:29–43. [PubMed: 1374023]

105. Sime PJ, Xing Z, Graham FL, Csaky KG, Gauldie J. Adenovector-mediated gene transfer of active transforming growth factor-beta1 induces prolonged severe fibrosis in rat lung. J Clin Invest. 1997; 100:768–776. [PubMed: 9259574]

106. Kang HR, Cho SJ, Lee CG, Homer RJ, Elias JA. Transforming growth factor (TGF)-beta1 stimulates pulmonary fibrosis and inflammation via a Bax-dependent, bid-activated pathway that involves matrix metalloproteinase-12. J Biol Chem. 2007; 282:7723–7732. [PubMed: 17209037]

107. Giri SN, Hyde DM, Hollinger MA. Effect of antibody to transforming growth factor beta on bleomycin induced accumulation of lung collagen in mice. Thorax. 1993; 48:959–966. [PubMed: 7504843]

108. Wang Q, Wang Y, Hyde DM, Gotwals PJ, Koteliansky VE, et al. Reduction of bleomycin induced lung fibrosis by transforming growth factor beta soluble receptor in hamsters. Thorax. 1999; 54:805–812. [PubMed: 10456973]

109. Feng XH, Derynck R. Specificity and versatility in tgf-beta signaling through Smads. Annu Rev Cell Dev Biol. 2005; 21:659–693. [PubMed: 16212511]

110. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature. 2003; 425:577–584. [PubMed: 14534577]

111. Lee MK, Pardoux C, Hall MC, Lee PS, Warburton D, et al. TGF-beta activates Erk MAP kinase signalling through direct phosphorylation of ShcA. Embo J. 2007; 26:3957–3967. [PubMed: 17673906]

112. Yamaguchi K, Shirakabe K, Shibuya H, Irie K, Oishi I, et al. Identification of a member of the MAPKKK family as a potential mediator of TGF-beta signal transduction. Science. 1995; 270:2008–2011. [PubMed: 8533096]

113. Shibuya H, Yamaguchi K, Shirakabe K, Tonegawa A, Gotoh Y, et al. TAB1: an activator of the TAK1 MAPKKK in TGF-beta signal transduction. Science. 1996; 272:1179–1182. [PubMed: 8638164]

114. Yamashita M, Fatyol K, Jin C, Wang X, Liu Z, et al. TRAF6 mediates Smad-independent activation of JNK and p38 by TGF-beta. Mol Cell. 2008; 31:918–924. [PubMed: 18922473]

115. Ge B, Gram H, Di Padova F, Huang B, New L, et al. MAPKK-independent activation of p38alpha mediated by TAB1-dependent autophosphorylation of p38alpha. Science. 2002; 295:1291–1294. [PubMed: 11847341]

116. Kato M, Zhang J, Wang M, Lanting L, Yuan H, et al. MicroRNA-192 in diabetic kidney glomeruli and its function in TGF-beta-induced collagen expression via inhibition of E-box repressors. Proc Natl Acad Sci U S A. 2007; 104:3432–3437. [PubMed: 17360662]

117. Kato M, Putta S, Wang M, Yuan H, Lanting L, et al. TGF-beta activates Akt kinase through a microRNA-dependent amplifying circuit targeting PTEN. Nat Cell Biol. 2009; 11:881–889. [PubMed: 19543271]

118. Bhowmick NA, Ghiassi M, Bakin A, Aakre M, Lundquist CA, et al. Transforming growth factor-beta1 mediates epithelial to mesenchymal transdifferentiation through a RhoA-dependent mechanism. Mol Biol Cell. 2001; 12:27–36. [PubMed: 11160820]

119. Smith PC, Caceres M, Martinez J. Induction of the myofibroblastic phenotype in human gingival fibroblasts by transforming growth factor-beta1: role of RhoA-ROCK and c-Jun N-terminal kinase signaling pathways. J Periodontal Res. 2006; 41:418–425. [PubMed: 16953819]

120. Lee J, Ko M, Joo CK. Rho plays a key role in TGF-beta1-induced cytoskeletal rearrangement in human retinal pigment epithelium. J Cell Physiol. 2008; 216:520–526. [PubMed: 18314880]

121. Masszi A, Di Ciano C, Sirokmany G, Arthur WT, Rotstein OD, et al. Central role for Rho in TGF-beta1-induced alpha-smooth muscle actin expression during epithelial-mesenchymal transition. Am J Physiol Renal Physiol. 2003; 284:F911–F924. [PubMed: 12505862]

122. Vardouli L, Moustakas A, Stournaras C. LIM-kinase 2 and cofilin phosphorylation mediate actin cytoskeleton reorganization induced by transforming growth factor-beta. J Biol Chem. 2005; 280:11448–11457. [PubMed: 15647284]

123. Rossman KL, Der CJ, Sondek J. GEF means go: turning on RHO GTPases with guanine nucleotide-exchange factors. Nat Rev Mol Cell Biol. 2005; 6:167–180. [PubMed: 15688002]

124. Yamanaka M, Shegogue D, Pei H, Bu S, Bielawska A, et al. Sphingosine kinase 1 (SPHK1) is induced by transforming growth factor-beta and mediates TIMP-1 up-regulation. J Biol Chem. 2004; 279:53994–54001. [PubMed: 15485866]

125. Kono Y, Nishiuma T, Nishimura Y, Kotani Y, Okada T, et al. Sphingosine kinase 1 regulates differentiation of human and mouse lung fibroblasts mediated by TGF-beta1. Am J Respir Cell Mol Biol. 2007; 37:395–404. [PubMed: 17641298]

126. Cencetti F, Bernacchioni C, Nincheri P, Donati C, Bruni P. Transforming growth factor-beta1 induces transdifferentiation of myoblasts into myofibroblasts via up-regulation of sphingosine kinase-1/S1P3 axis. Mol Biol Cell. 21:1111–1124. [PubMed: 20089836]

127. Medlin MD, Staus DP, Dubash AD, Taylor JM, Mack CP. Sphingosine 1-phosphate receptor 2 signals through leukemia-associated RhoGEF (LARG), to promote smooth muscle cell differentiation. Arterioscler Thromb Vasc Biol. 30:1779–1786. [PubMed: 20702813]

128. Shen X, Li J, Hu PP, Waddell D, Zhang J, et al. The activity of guanine exchange factor NET1 is essential for transforming growth factor-beta-mediated stress fiber formation. J Biol Chem. 2001; 276:15362–15368. [PubMed: 11278519]

129. Lee J, Moon HJ, Lee JM, Joo CK. Smad3 regulates Rho signaling via NET1 in the transforming growth factor-beta-induced epithelial-mesenchymal transition of human retinal pigment epithelial cells. J Biol Chem. 285:26618–26627. [PubMed: 20547485]

130. Tsapara A, Luthert P, Greenwood J, Hill CS, Matter K, et al. The RhoA activator GEF-H1/Lfc is a transforming growth factor-beta target gene and effector that regulates alpha-smooth muscle actin expression and cell migration. Mol Biol Cell. 21:860–870. [PubMed: 20089843]

131. Delanoe-Ayari H, Al Kurdi R, Vallade M, Gulino-Debrac D, Riveline D. Membrane and acto-myosin tension promote clustering of adhesion proteins. Proc Natl Acad Sci U S A. 2004; 101:2229–2234. [PubMed: 14982992]

132. Domnina LV, Gelfand VI, Ivanova OY, Leonova EV, Pletjushkina OY, et al. Effects of small doses of cytochalasins on fibroblasts: preferential changes of active edges and focal contacts. Proc Natl Acad Sci U S A. 1982; 79:7754–7757. [PubMed: 6961447]

133. del Rio A, Perez-Jimenez R, Liu R, Roca-Cusachs P, Fernandez JM, et al. Stretching single talin rod molecules activates vinculin binding. Science. 2009; 323:638–641. [PubMed: 19179532]

134. Geiger B, Spatz JP, Bershadsky AD. Environmental sensing through focal adhesions. Nat Rev Mol Cell Biol. 2009; 10:21–33. [PubMed: 19197329]

135. Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. Science. 2005; 310:1139–1143. [PubMed: 16293750]
136. Vogel V, Sheetz M. Local force and geometry sensing regulate cell functions. Nat Rev Mol Cell Biol. 2006; 7:265–275. [PubMed: 16607289]
137. Giannone G, Sheetz MP. Substrate rigidity and force define form through tyrosine phosphatase and kinase pathways. Trends Cell Biol. 2006; 16:213–223. [PubMed: 16529933]
138. Sawada Y, Sheetz MP. Force transduction by Triton cytoskeletons. J Cell Biol. 2002; 156:609–615. [PubMed: 11839769]
139. Schoenwaelder SM, Burridge K. Bidirectional signaling between the cytoskeleton and integrins. Curr Opin Cell Biol. 1999; 11:274–286. [PubMed: 10209151]
140. Han B, Bai XH, Lodyga M, Xu J, Yang BB, et al. Conversion of mechanical force into biochemical signaling. J Biol Chem. 2004; 279:54793–54801. [PubMed: 15485829]
141. Nobes CD, Hawkins P, Stephens L, Hall A. Activation of the small GTP-binding proteins rho and rac by growth factor receptors. J Cell Sci. 1995; 108(Pt 1):225–233. [PubMed: 7738099]
142. Schoenwaelder SM, Petch LA, Williamson D, Shen R, Feng GS, et al. The protein tyrosine phosphatase Shp-2 regulates RhoA activity. Curr Biol. 2000; 10:1523–1526. [PubMed: 11114521]
143. Tamada M, Sheetz MP, Sawada Y. Activation of a signaling cascade by cytoskeleton stretch. Dev Cell. 2004; 7:709–718. [PubMed: 15525532]
144. Romer LH, Birukov KG, Garcia JG. Focal adhesions: paradigm for a signaling nexus. Circ Res. 2006; 98:606–616. [PubMed: 16543511]
145. Tilghman RW, Parsons JT. Focal adhesion kinase as a regulator of cell tension in the progression of cancer. Semin Cancer Biol. 2008; 18:45–52. [PubMed: 17928235]
146. Mao Y, Schwarzbauer JE. Fibronectin fibrillogenesis, a cell-mediated matrix assembly process. Matrix Biol. 2005; 24:389–399. [PubMed: 16061370]
147. Wierzbicka-Patynowski I, Schwarzbauer JE. The ins and outs of fibronectin matrix assembly. J Cell Sci. 2003; 116:3269–3276. [PubMed: 12857786]
148. Ohashi T, Kiehart DP, Erickson HP. Dynamics and elasticity of the fibronectin matrix in living cell culture visualized by fibronectin-green fluorescent protein. Proc Natl Acad Sci U S A. 1999; 96:2153–2158. [PubMed: 10051610]
149. Fogerty FJ, Akiyama SK, Yamada KM, Mosher DF. Inhibition of binding of fibronectin to matrix assembly sites by anti-integrin (alpha 5 beta 1) antibodies. J Cell Biol. 1990; 111:699–708. [PubMed: 2380248]
150. Miranti CK, Brugge JS. Sensing the environment: a historical perspective on integrin signal transduction. Nat Cell Biol. 2002; 4:E83–E90. [PubMed: 11944041]
151. McKeown-Longo PJ, Mosher DF. Binding of plasma fibronectin to cell layers of human skin fibroblasts. J Cell Biol. 1983; 97:466–472. [PubMed: 6309861]
152. Wu C, Keivens VM, O'Toole TE, McDonald JA, Ginsberg MH. Integrin activation and cytoskeletal interaction are essential for the assembly of a fibronectin matrix. Cell. 1995; 83:715–724. [PubMed: 8521488]
153. Hynes RO, Destree AT. Relationships between fibronectin (LETS protein) and actin. Cell. 1978; 15:875–886. [PubMed: 365353]
154. Ali IU, Hynes RO. Effects of cytochalasin B and colchicine on attachment of a major surface protein of fibroblasts. Biochim Biophys Acta. 1977; 471:16–24. [PubMed: 921971]
155. Zhang Q, Magnusson MK, Mosher DF. Lysophosphatidic acid and microtubule-destabilizing agents stimulate fibronectin matrix assembly through Rho-dependent actin stress fiber formation and cell contraction. Mol Biol Cell. 1997; 8:1415–1425. [PubMed: 9285815]
156. Zhong C, Chrzanowska-Wodnicka M, Brown J, Shaub A, Belkin AM, et al. Rho-mediated contractility exposes a cryptic site in fibronectin and induces fibronectin matrix assembly. J Cell Biol. 1998; 141:539–551. [PubMed: 9548730]
157. Baneyx G, Baugh L, Vogel V. Fibronectin extension and unfolding within cell matrix fibrils controlled by cytoskeletal tension. Proc Natl Acad Sci U S A. 2002; 99:5139–5143. [PubMed: 11959962]

158. Midwood KS, Schwarzbauer JE. Tenascin-C modulates matrix contraction via focal adhesion kinase- and Rho-mediated signaling pathways. Mol Biol Cell. 2002; 13:3601–3613. [PubMed: 12388760]

159. Saoncella S, Echtermeyer F, Denhez F, Nowlen JK, Mosher DF, et al. Syndecan-4 signals cooperatively with integrins in a Rho-dependent manner in the assembly of focal adhesions and actin stress fibers. Proc Natl Acad Sci U S A. 1999; 96:2805–2810. [PubMed: 10077592]

160. Halliday NL, Tomasek JJ. Mechanical properties of the extracellular matrix influence fibronectin fibril assembly in vitro. Exp Cell Res. 1995; 217:109–117. [PubMed: 7867709]

161. Pankov R, Cukierman E, Katz BZ, Matsumoto K, Lin DC, et al. Integrin dynamics and matrix assembly: tensin-dependent translocation of alpha(5)beta(1) integrins promotes early fibronectin fibrillogenesis. J Cell Biol. 2000; 148:1075–1090. [PubMed: 10704455]

162. Peters DM, Mosher DF. Localization of cell surface sites involved in fibronectin fibrillogenesis. J Cell Biol. 1987; 104:121–130. [PubMed: 2947903]

163. Zamir E, Katz M, Posen Y, Erez N, Yamada KM, et al. Dynamics and segregation of cell-matrix adhesions in cultured fibroblasts. Nat Cell Biol. 2000; 2:191–196. [PubMed: 10783236]

164. Ohashi T, Kiehart DP, Erickson HP. Dual labeling of the fibronectin matrix and actin cytoskeleton with green fluorescent protein variants. J Cell Sci. 2002; 115:1221–1229. [PubMed: 11884521]

165. Harris AK, Stopak D, Wild P. Fibroblast traction as a mechanism for collagen morphogenesis. Nature. 1981; 290:249–251. [PubMed: 7207616]

166. Kolodney MS, Wysolmerski RB. Isometric contraction by fibroblasts and endothelial cells in tissue culture: a quantitative study. J Cell Biol. 1992; 117:73–82. [PubMed: 1556157]

167. Isenberg G, Rathke PC, Hulsmann N, Franke WW, Wohlfarth-Bottermann KE. Cytoplasmic actomyosin fibrils in tissue culture cells: direct proof of contractility by visualization of ATP-induced contraction in fibrils isolated by laser micro-beam dissection. Cell Tissue Res. 1976; 166:427–443. [PubMed: 1253242]

168. Kreis TE, Birchmeier W. Stress fiber sarcomeres of fibroblasts are contractile. Cell. 1980; 22:555–561. [PubMed: 6893813]

169. Katoh K, Kano Y, Masuda M, Onishi H, Fujiwara K. Isolation and contraction of the stress fiber. Mol Biol Cell. 1998; 9:1919–1938. [PubMed: 9658180]

170. Hinz B, Celetta G, Tomasek JJ, Gabbiani G, Chaponnier C. Alpha-smooth muscle actin expression upregulates fibroblast contractile activity. Mol Biol Cell. 2001; 12:2730–2741. [PubMed: 11553712]

171. Burridge K. Are stress fibres contractile? Nature. 1981; 294:691–692. [PubMed: 7198718]

172. Nobes CD, Hall A. Rho GTPases control polarity, protrusion, and adhesion during cell movement. J Cell Biol. 1999; 144:1235–1244. [PubMed: 10087266]

173. Totsukawa G, Wu Y, Sasaki Y, Hartshorne DJ, Yamakita Y, et al. Distinct roles of MLCK and ROCK in the regulation of membrane protrusions and focal adhesion dynamics during cell migration of fibroblasts. J Cell Biol. 2004; 164:427–439. [PubMed: 14757754]

174. Jay PY, Pham PA, Wong SA, Elson EL. A mechanical function of myosin II in cell motility. J Cell Sci. 1995; 108(Pt 1):387–393. [PubMed: 7738114]

175. Kolega J. Cytoplasmic dynamics of myosin IIA and IIB: spatial 'sorting' of isoforms in locomoting cells. J Cell Sci. 1998; 111(Pt 15):2085–2095. [PubMed: 9664030]

176. Alblas J, Ulfman L, Hordijk P, Koenderman L. Activation of Rhoa and ROCK are essential for detachment of migrating leukocytes. Mol Biol Cell. 2001; 12:2137–2145. [PubMed: 11452009]

177. Worthylake RA, Lemoine S, Watson JM, Burridge K. RhoA is required for monocyte tail retraction during transendothelial migration. J Cell Biol. 2001; 154:147–160. [PubMed: 11448997]

178. Cramer LP, Siebert M, Mitchison TJ. Identification of novel graded polarity actin filament bundles in locomoting heart fibroblasts: implications for the generation of motile force. J Cell Biol. 1997; 136:1287–1305. [PubMed: 9087444]

179. Oliver T, Lee J, Jacobson K. Forces exerted by locomoting cells. Semin Cell Biol. 1994; 5:139–147. [PubMed: 7919227]

180. Huttelmaier S, Zenklusen D, Lederer M, Dictenberg J, Lorenz M, et al. Spatial regulation of beta-actin translation by Src-dependent phosphorylation of ZBP1. Nature. 2005; 438:512–515. [PubMed: 16306994]

181. Rodriguez AJ, Czaplinski K, Condeelis JS, Singer RH. Mechanisms and cellular roles of local protein synthesis in mammalian cells. Curr Opin Cell Biol. 2008; 20:144–149. [PubMed: 18378131]

182. Lawrence JB, Singer RH. Intracellular localization of messenger RNAs for cytoskeletal proteins. Cell. 1986; 45:407–415. [PubMed: 3698103]

183. Kislauskis EH, Singer RH. Determinants of mRNA localization. Curr Opin Cell Biol. 1992; 4:975–978. [PubMed: 1485968]

184. Latham VM Jr, Kislauskis EH, Singer RH, Ross AF. Beta-actin mRNA localization is regulated by signal transduction mechanisms. J Cell Biol. 1994; 126:1211–1219. [PubMed: 8063858]

185. Kislauskis EH, Zhu X, Singer RH. Sequences responsible for intracellular localization of beta-actin messenger RNA also affect cell phenotype. J Cell Biol. 1994; 127:441–451. [PubMed: 7929587]

186. Bassell G, Singer RH. mRNA and cytoskeletal filaments. Curr Opin Cell Biol. 1997; 9:109–115. [PubMed: 9013679]

187. Lenk R, Ransom L, Kaufmann Y, Penman S. A cytoskeletal structure with associated polyribosomes obtained from HeLa cells. Cell. 1977; 10:67–78. [PubMed: 837445]

188. Fusco D, Accornero N, Lavoie B, Shenoy SM, Blanchard JM, et al. Single mRNA molecules demonstrate probabilistic movement in living mammalian cells. Curr Biol. 2003; 13:161–167. [PubMed: 12546792]

189. Sundell CL, Singer RH. Requirement of microfilaments in sorting of actin messenger RNA. Science. 1991; 253:1275–1277. [PubMed: 1891715]

190. Taneja KL, Lifshitz LM, Fay FS, Singer RH. Poly(A) RNA codistribution with microfilaments: evaluation by in situ hybridization and quantitative digital imaging microscopy. J Cell Biol. 1992; 119:1245–1260. [PubMed: 1360014]

191. Latham VM, Yu EH, Tullio AN, Adelstein RS, Singer RH. A Rho-dependent signaling pathway operating through myosin localizes beta-actin mRNA in fibroblasts. Curr Biol. 2001; 11:1010–1016. [PubMed: 11470405]

192. Ornelles DA, Fey EG, Penman S. Cytochalasin releases mRNA from the cytoskeletal framework and inhibits protein synthesis. Mol Cell Biol. 1986; 6:1650–1662. [PubMed: 3785175]

193. Chicurel ME, Singer RH, Meyer CJ, Ingber DE. Integrin binding and mechanical tension induce movement of mRNA and ribosomes to focal adhesions. Nature. 1998; 392:730–733. [PubMed: 9565036]

194. Hill MA, Schedlich L, Gunning P. Serum-induced signal transduction determines the peripheral location of beta-actin mRNA within the cell. J Cell Biol. 1994; 126:1221–1229. [PubMed: 8063859]

195. Toh BH, Lolait SJ, Mathy JP, Baum R. Association of mitochondria with intermediate filaments and of polyribosomes with cytoplasmic actin. Cell Tissue Res. 1980; 211:163–169. [PubMed: 6996829]

196. Heuser JE, Kirschner MW. Filament organization revealed in platinum replicas of freeze-dried cytoskeletons. J Cell Biol. 1980; 86:212–234. [PubMed: 6893451]

197. Hesketh JE, Horne Z, Campbell GP. Immunohistochemical evidence for an association of ribosomes with microfilaments in 3T3 fibroblasts. Cell Biol Int Rep. 1991; 15:141–150. [PubMed: 2029732]

198. Zumbe A, Stahli C, Trachsel H. Association of a Mr 50,000 cap-binding protein with the cytoskeleton in baby hamster kidney cells. Proc Natl Acad Sci U S A. 1982; 79:2927–2931. [PubMed: 7045875]

199. Heuijerjans JH, Pieper FR, Ramaekers FC, Timmermans LJ, Kuijpers H, et al. Association of mRNA and eIF-2 alpha with the cytoskeleton in cells lacking vimentin. Exp Cell Res. 1989; 181:317–330. [PubMed: 2466674]

200. Edmonds BT, Wyckoff J, Yeung YG, Wang Y, Stanley ER, et al. Elongation factor-1 alpha is an overexpressed actin binding protein in metastatic rat mammary adenocarcinoma. J Cell Sci. 1996; 109(Pt 11):2705–2714. [PubMed: 8937988]

201. Shestakova EA, Wyckoff J, Jones J, Singer RH, Condeelis J. Correlation of beta-actin messenger RNA localization with metastatic potential in rat adenocarcinoma cell lines. Cancer Res. 1999; 59:1202–1205. [PubMed: 10096548]

202. Liu G, Grant WM, Persky D, Latham VM Jr, Singer RH, et al. Interactions of elongation factor 1alpha with F-actin and beta-actin mRNA: implications for anchoring mRNA in cell protrusions. Mol Biol Cell. 2002; 13:579–592. [PubMed: 11854414]

203. Pittman YR, Kandl K, Lewis M, Valente L, Kinzy TG. Coordination of eukaryotic translation elongation factor 1A (eEF1A) function in actin organization and translation elongation by the guanine nucleotide exchange factor eEF1Balpha. J Biol Chem. 2009; 284:4739–4747. [PubMed: 19095653]

204. Mateyak MK, Kinzy TG. eEF1A: thinking outside the ribosome. J Biol Chem. 2010; 285:21209–21213. [PubMed: 20444696]

205. Hesketh JE, Pryme IF. Interaction between mRNA, ribosomes and the cytoskeleton. Biochem J. 1991; 277(Pt 1):1–10. [PubMed: 1854327]

206. Condeelis J, Singer RH. How and why does beta-actin mRNA target? Biol Cell. 2005; 97:97–110. [PubMed: 15601261]

207. Mingle LA, Okuhama NN, Shi J, Singer RH, Condeelis J, et al. Localization of all seven messenger RNAs for the actin-polymerization nucleator Arp2/3 complex in the protrusions of fibroblasts. J Cell Sci. 2005; 118:2425–2433. [PubMed: 15923655]

208. Rodriguez AJ, Shenoy SM, Singer RH, Condeelis J. Visualization of mRNA translation in living cells. J Cell Biol. 2006; 175:67–76. [PubMed: 17030983]

209. Shestakova EA, Singer RH, Condeelis J. The physiological significance of beta -actin mRNA localization in determining cell polarity and directional motility. Proc Natl Acad Sci U S A. 2001; 98:7045–7050. [PubMed: 11416185]

210. Wang W, Wyckoff JB, Frohlich VC, Oleynikov Y, Huttelmaier S, et al. Single cell behavior in metastatic primary mammary tumors correlated with gene expression patterns revealed by molecular profiling. Cancer Res. 2002; 62:6278–6288. [PubMed: 12414658]

211. Wyckoff JB, Jones JG, Condeelis JS, Segall JE. A critical step in metastasis: in vivo analysis of intravasation at the primary tumor. Cancer Res. 2000; 60:2504–2511. [PubMed: 10811132]

212. Lapidus K, Wyckoff J, Mouneimne G, Lorenz M, Soon L, et al. ZBP1 enhances cell polarity and reduces chemotaxis. J Cell Sci. 2007; 120:3173–3178. [PubMed: 17878234]

213. Miano JM. Serum response factor: toggling between disparate programs of gene expression. J Mol Cell Cardiol. 2003; 35:577–593. [PubMed: 12788374]

214. Hill CS, Wynne J, Treisman R. The Rho family GTPases RhoA, Rac1, and CDC42Hs regulate transcriptional activation by SRF. Cell. 1995; 81:1159–1170. [PubMed: 7600583]

215. Sotiropoulos A, Gineitis D, Copeland J, Treisman R. Signal-regulated activation of serum response factor is mediated by changes in actin dynamics. Cell. 1999; 98:159–169. [PubMed: 10428028]

216. Miralles F, Posern G, Zaromytidou AI, Treisman R. Actin dynamics control SRF activity by regulation of its coactivator MAL. Cell. 2003; 113:329–342. [PubMed: 12732141]

217. Descot A, Hoffmann R, Shaposhnikov D, Reschke M, Ullrich A, et al. Negative regulation of the EGFR-MAPK cascade by actin-MAL-mediated Mig6/Errfi-1 induction. Mol Cell. 2009; 35:291–304. [PubMed: 19683494]

218. Lockman K, Hinson JS, Medlin MD, Morris D, Taylor JM, et al. Sphingosine 1-phosphate stimulates smooth muscle cell differentiation and proliferation by activating separate serum response factor co-factors. J Biol Chem. 2004; 279:42422–42430. [PubMed: 15292266]

219. Gohla A, Offermanns S, Wilkie TM, Schultz G. Differential involvement of Galpha12 and Galpha13 in receptor-mediated stress fiber formation. J Biol Chem. 1999; 274:17901–17907. [PubMed: 10364236]

220. Zhao XH, Laschinger C, Arora P, Szaszi K, Kapus A, et al. Force activates smooth muscle alpha-actin promoter activity through the Rho signaling pathway. J Cell Sci. 2007; 120:1801–1809. [PubMed: 17456553]

221. Small EM, Thatcher JE, Sutherland LB, Kinoshita H, Gerard RD, et al. Myocardin-related transcription factor-a controls myofibroblast activation and fibrosis in response to myocardial infarction. Circ Res. 107:294–304. [PubMed: 20558820]

222. Hinson JS, Medlin MD, Lockman K, Taylor JM, Mack CP. Smooth muscle cell-specific transcription is regulated by nuclear localization of the myocardin-related transcription factors. Am J Physiol Heart Circ Physiol. 2007; 292:H1170–H1180. [PubMed: 16997888]

223. Sandbo N, Kregel S, Taurin S, Bhorade S, Dulin NO. Critical role of serum response factor in pulmonary myofibroblast differentiation induced by TGF-beta. Am J Respir Cell Mol Biol. 2009; 41:332–338. [PubMed: 19151320]

224. Fan L, Sebe A, Peterfi Z, Masszi A, Thirone AC, et al. Cell contact-dependent regulation of epithelial-myofibroblast transition via the rho-rho kinase-phospho-myosin pathway. Mol Biol Cell. 2007; 18:1083–1097. [PubMed: 17215519]

225. Morita T, Mayanagi T, Sobue K. Dual roles of myocardin-related transcription factors in epithelial mesenchymal transition via slug induction and actin remodeling. J Cell Biol. 2007; 179:1027–1042. [PubMed: 18056415]

226. Sun Q, Chen G, Streb JW, Long X, Yang Y, et al. Defining the mammalian CArGome. Genome Res. 2006; 16:197–207. [PubMed: 16365378]

227. Morita T, Mayanagi T, Sobue K. Reorganization of the actin cytoskeleton via transcriptional regulation of cytoskeletal/focal adhesion genes by myocardin-related transcription factors (MRTFs/MAL/MKLs). Exp Cell Res. 2007; 313:3432–3445. [PubMed: 17714703]

228. Philimonenko VV, Zhao J, Iben S, Dingova H, Kysela K, et al. Nuclear actin and myosin I are required for RNA polymerase I transcription. Nat Cell Biol. 2004; 6:1165–1172. [PubMed: 15558034]

229. Hofmann WA, Stojiljkovic L, Fuchsova B, Vargas GM, Mavrommatis E, et al. Actin is part of pre-initiation complexes and is necessary for transcription by RNA polymerase II. Nat Cell Biol. 2004; 6:1094–1101. [PubMed: 15502823]

230. Visa N. Actin in transcription. Actin is required for transcription by all three RNA polymerases in the eukaryotic cell nucleus. EMBO Rep. 2005; 6:218–219. [PubMed: 15741974]

231. Vartiainen MK. Nuclear actin dynamics--from form to function. FEBS Lett. 2008; 582:2033–2040. [PubMed: 18423404]

232. Kaufman LJ, Brangwynne CP, Kasza KE, Filippidi E, Gordon VD, et al. Glioma expansion in collagen I matrices: analyzing collagen concentration-dependent growth and motility patterns. Biophys J. 2005; 89:635–650. [PubMed: 15849239]

233. Rizvi SA, Neidt EM, Cui J, Feiger Z, Skau CT, et al. Identification and characterization of a small molecule inhibitor of formin-mediated actin assembly. Chem Biol. 2009; 16:1158–1168. [PubMed: 19942139]

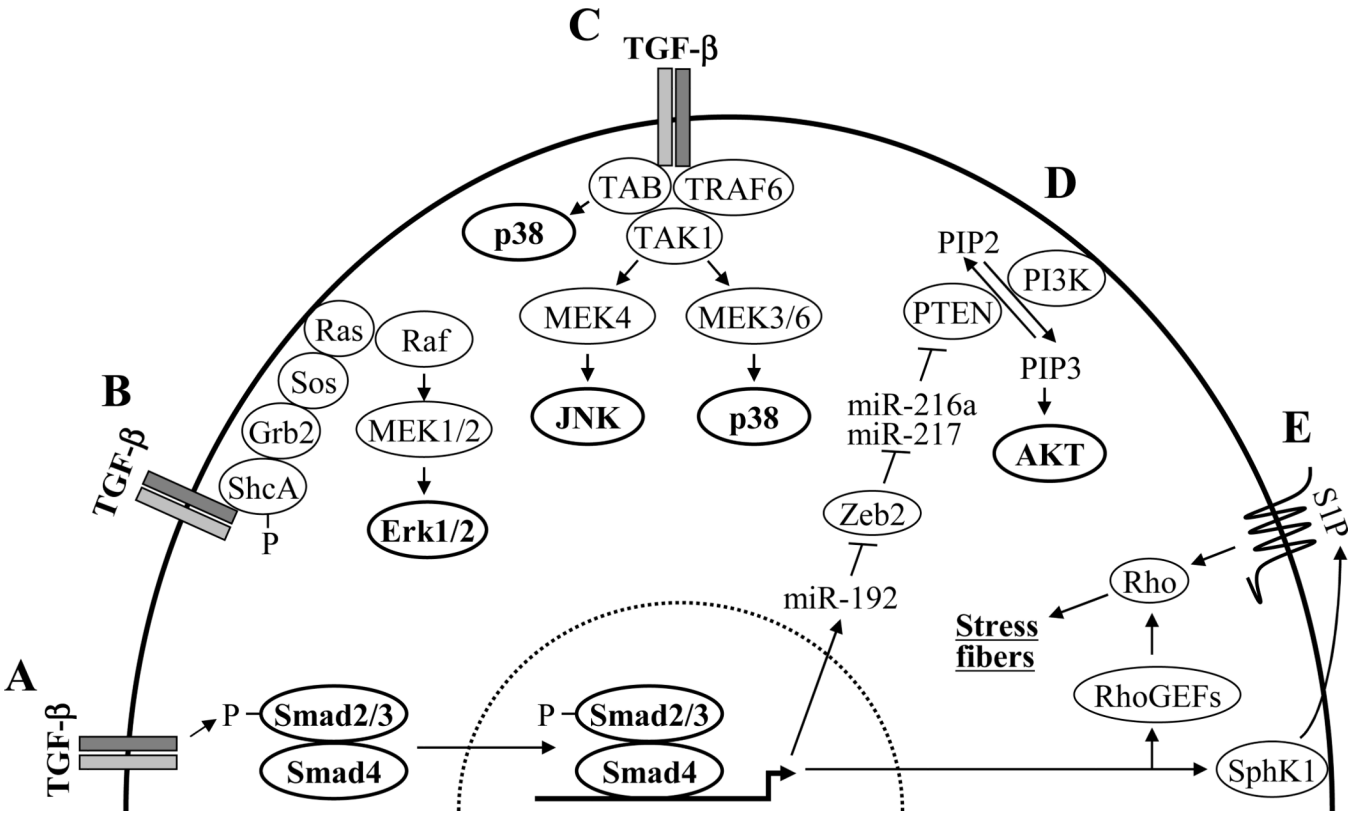

Figure 1.

TGF-β signaling through Smads (A) and non-canonical signaling leading to activation of ERK (B), JNK and p38 MAP kinase (C), AKT (D) and Rho/stress fiber formation (E).

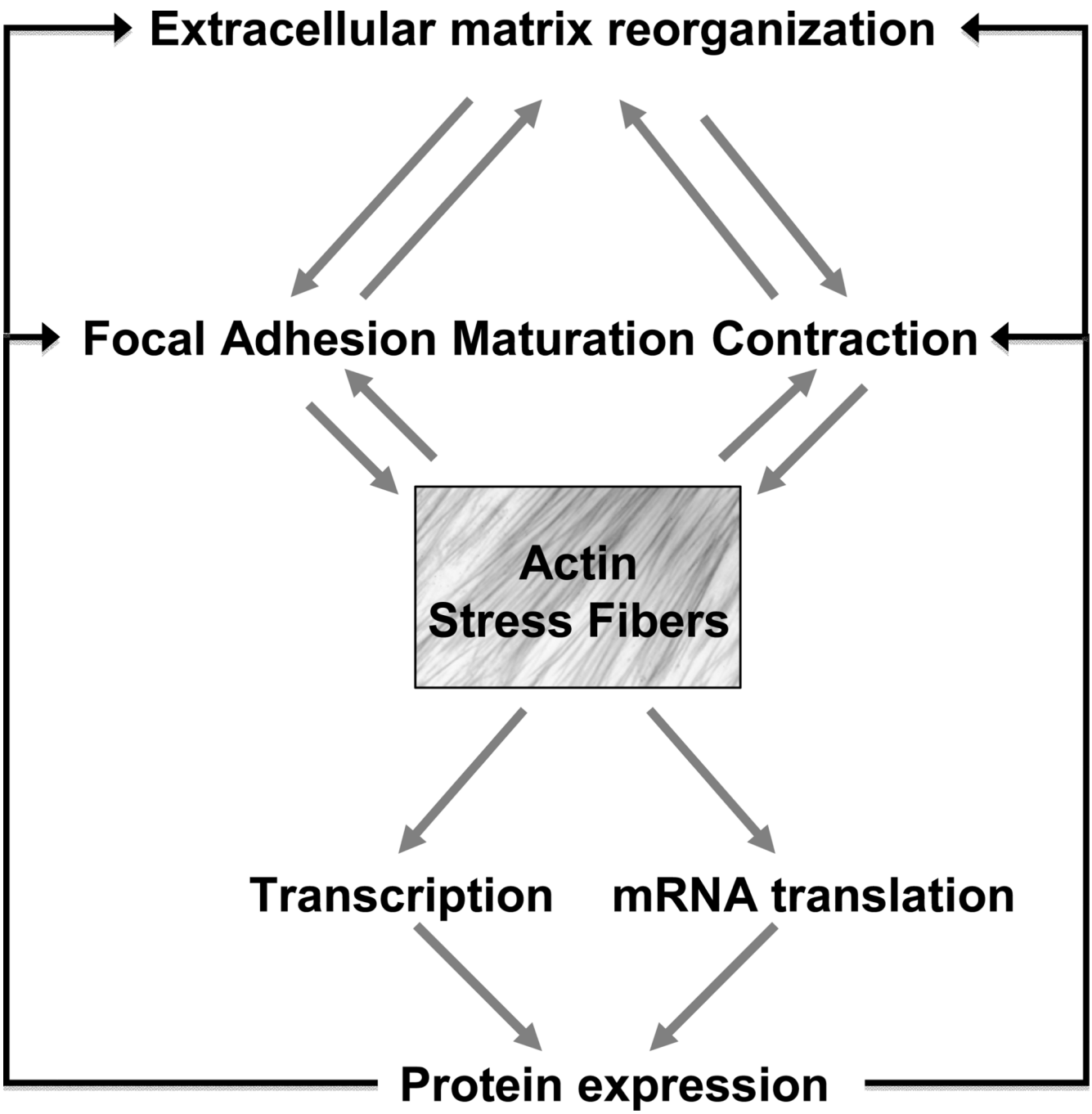

Figure 2.
The role of the actin cytoskeleton in modulating myofibroblast functions. The actin cytoskeleton regulates several mechanical functions during myofibroblast differentiation (focal adhesion formation, contraction, and matrix remodeling), but simultaneously controls the transcription and translation of several genes that are involved in these same mechanical functions. In this way, the actin stress fiber plays an important role in amplifying the signals leading to myofibroblast differentiation. This feedback is bidirectional, as matrix stiffness, focal adhesion formation and contractility all are stimuli for augmented stress fiber formation.