Mechanical stress regulates gene expression via Rho/Rho-kinase signaling pathway

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Abstract Exercise improves the performance of the cardiovascular system, striated muscle, and bones, and reduces obesity. Exercise provides for mechanical forces, which include fluid flow and stretching. These mechanical stimuli alter gene expression, which induces the physiological and pathological response in the organs and cells. However, the molecular mechanism underlying the conversion of mechanical stress into gene expression remains largely unknown. Recently, it has been reported that the mechanical stimuli alter the localization of the transcriptional co-activators MRTF (myocardin-related transcription factor) and YAP (Yes-associated protein)/TAZ (transcriptional co-activator with PDZ-binding motif) through actin remodeling. Since MRTF and YAP/TAZ are retained in the cytoplasm in a monomeric G (globular)-actin-dependent manner, actin polymerization promotes the translocation of MRTF and YAP/TAZ to the nucleus, and regulates the expression of several genes. The Rho family small GTPase member Rho and its effector, Rho-kinase (Rho-associated kinase), are critical regulators of actin remodeling. Rho/Rho-kinase suppresses the severing of actin through LIMK (LIM-kinase) and cofilin phosphorylation, thus enhancing actin polymerization. Mechanical stimuli induce actin polymerization through the Rho/Rho-kinase-LIMK-cofilin pathway. The Rho/Rho-kinase signaling pathway regulates gene expression and cell lineage by controlling the actin-dependent localization of MRTF and YAP/TAZ in response to mechanical stress. In this review, we focus on the molecular mechanism by which Rho/Rho-kinase controls gene expression in response to mechanical stimuli.

Keywords : actin, mechanotransduction, MRTF, Rho, Rho-kinase, YAP/TAZ

Introduction

Mechanotransduction is the cellular process that translates mechanical stimuli into biochemical signals, enabling cells to adapt to their physical surroundings¹⁾. Mechanical stimuli include fluid flow, substrate strain, membrane deformation, vibration, altered gravity, and compressive loading (increased hydrostatic pressure)²⁾. These mechanical stimuli regulate cell and tissue physiology by regulating gene expression. Mechanotransduction signaling has an important role in the maintenance of several mechanically stressed tissues, such as muscle, bone, cartilage, and blood vessels¹⁾. Stem cells are induced toward specific fates during differentiation on the basis of the geometry and stiffness of the substrate on which they are grown¹⁾. Mechanotransduction is required for developmental pathways and normal tissue homeostasis. Exercise induces the hypertrophic growth of skeletal and cardiac muscle, whereas immobilized muscles gradually atrophy¹⁾. Excessive load induces pathological hypertrophy accompanied by cardiac failure¹⁾. Gravity and compressive forces in-

duce bone remodeling and optimize the physical performance of bone¹⁾, whereas bone is lost when mechanical forces are decreased, for example, during space travel, bed rest, or spinal cord injury³⁾. Therefore, mechanical stimuli are required for the physiological and pathological responses in cells and tissues. However, the molecular mechanisms of mechanotransduction are still poorly understood.

The small GTPase, RhoA, and its effector, Rho-associated kinase (Rho-kinase), are associated with mechanotransduction. Exogenous forces lead to RhoA activation in different cell types^{4,5)}. Excessive load leads to pathological hypertrophy, which is associated with the Rho/Rho-kinase signaling pathway⁶⁾. Rho/Rho-kinase is known as a key regulator of actin polymerization. Recently, it has been shown that the Rho/Rho-kinase signaling pathway regulates gene expression in response to mechanical stress through the remodeling of the actin cytoskeleton. In this review, we will focus on the molecular mechanisms underlying the regulation of gene expression in response to mechanical stimuli by the Rho/Rho-kinase signaling pathway.

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Rho/Rho-kinase regulates actin formation

The Rho family GTPase member, Rho, acts as a molecular switch, cycling between an active GTP-bound and an inactive GDP-bound form⁶. Conversion from the inactive form to the active form is regulated by guanine nucleotide exchange factors (GEFs), which facilitate the exchange of GDP for GTP. GTPase-activating proteins (GAPs) enhance the intrinsic rate of GTP hydrolysis by Rho GTPase (Fig. 1). Guanine nucleotide dissociation inhibitors (GDIs) associate with inactivated Rho and maintain the GDP-bound inactive form of Rho. RhoA is one of the most extensively characterized members of the Rho

family small GTPases. The activation and inactivation of RhoA is modulated both by intracellular and extracellular signals from G protein-coupled receptors, integrins, and growth factor receptors. The activation of RhoA induces the formation of actin stress fibers, actin polymerization, and focal adhesion⁶. RhoA exerts its biological functions through association with specific effectors⁷ such as Rho-kinase and mammalian Diaphanous (mDia), which are known as the major effectors of RhoA⁷⁻¹⁰.

Rho-kinase is identified as an effector of RhoA, and RhoA activates Rho-kinase downstream of various extracellular signals¹¹⁻¹³. There are two Rho-kinase members, ROCK1 (known as Rho-kinase β or ROK β) and ROCK2

Fig. 1 Rho/Rho-kinase regulates actin polymerization

Mechanical stimuli activate the Rho/Rho-kinase signaling pathway. The small GTPase RhoA switches between a GTP-bound active form (mediated by RhoGEF [guanine nucleotide exchange factor]) and a GDP-bound inactive form (mediated by RhoGAP [GTPase-activating protein]). Activated RhoA activates Rho-kinase, which in turn phosphorylates and activates LIMK (LIM-kinase). Cofilin enhances actin depolymerization through its severing activity. Cofilin is phosphorylated and inactivated by LIMK. Therefore, Rho/Rho-kinase enhances F-actin formation through LIMK and cofilin. mDia (mammalian Diaphanous-related formin) is also an effector of RhoA, and RhoA induces actin polymerization through mDia. Rho-kinase enhances the phosphorylation of MLC (myosin light chain) through its direct phosphorylation, and the suppression of MLCP (myosin light chain phosphatase). Thus, Rho/Rho-kinase enhances actomyosin contraction through the phosphorylation of MLC.

(known as Rho-kinase α or ROK α); they are collectively referred to as Rho-kinase hereafter^{14,15}).

Cytoplasmic actin dynamics cause changes in both the globular actin (G-actin) concentrations and the filamentous actin (F-actin) structure¹⁶. RhoA promotes actin polymerization through Rho-kinase¹⁵. In mammals, the actin-depolymerizing factor (ADF)/cofilin family of proteins consists of cofilin-1, cofilin-2, and ADF (also known as destrin)¹⁷. Cofilin-1 is not found in muscles and is known as a non-muscle type of cofilin, while cofilin-2 is found in muscles and is known as a muscle type of cofilin¹⁷. Cofilin associates with both G-actin and F-actin, and plays a crucial role in actin filament dynamics and reorganization by stimulating actin filament severing and depolymerization¹⁷. Cofilin activity—actin binding, severing, and depolymerization—is inhibited by the phosphorylation of its serine 3 (Ser-3) residue by LIM-kinase (LIMK)¹⁸⁻²⁰. There are two LIMKs in mammals, namely, LIMK1 and LIMK2. Rho-kinase phosphorylates and activates LIMKs²¹⁻²⁴, and therefore, the Rho/Rho-kinase signaling pathway enhances actin polymerization by inhibiting cofilin activity through LIMK (Fig. 1).

Rho-kinase enhances actomyosin contractility through the phosphorylation of myosin light chain. Additionally, Rho-kinase suppresses the activity of myosin light chain phosphatase, leading to stress fiber formation (Fig. 1)²⁵⁻²⁷. RhoA also accelerates actin polymerization through mDia, which interacts with the barbed end of actin filaments and promotes actin polymerization (Fig. 1)²⁸. The formation of actin stress fibers is regulated by mDia and Rho-kinase downstream of RhoA²⁹. Mechanical stress induces actin polymerization through the Rho/Rho-kinase-LIMK-cofilin pathway³⁰. It has also been reported that mDia is required for actin assembly at sites of mechanical force³¹.

Several recent studies have suggested that mechanical stimuli induce the translocation of the transcriptional co-activators myocardin-related transcription factor (MRTF) and Yes-associated protein (YAP)/transcriptional co-activator with PDZ-binding motif (TAZ), and enhance the expression of several genes. Rho/Rho-kinase translates mechanical stimuli into gene expression changes through the actin-MRTF-SRF pathway³⁰. Additionally, it has been reported that stretch stimulation induces YAP/TAZ activation and nucleus accumulation through Rho/Rho-kinase dependent actin rearrangement³². Here, we focus on two major actin-dependent mechanotransduction pathways, namely, MRTF and YAP/TAZ, which are modulated by Rho/Rho-kinase.

Rho/Rho-kinase regulates gene expression through MRTF-SRF in response to mechanical stimuli

The concentration of actin monomers regulates serum response factor (SRF) activity³³, and SRF directs the transcription of numerous target genes upon stimula-

tion by various signaling cascades, including mechanical stress^{16,30}. RhoA is activated during the early stages of endothelial actin remodeling induced by shear stress⁵. Stretch forces induce RhoA activation through integrins and increase actin filament assembly³⁰.

MRTF, also known as MAL, BSAC or MKL, is a transcriptional co-activator that regulates SRF binding to the serum response element-binding sites, the CArG-boxes¹⁶. The MRTF family consists of myocardin, MRTF-A (also known as MAL, MKL1, and BSAC), and MRTF-B (MKL2 and MAL16)¹⁶. Myocardin is expressed in the cardiovascular system, while other MRTF family members exhibit a more ubiquitous expression pattern³⁴. The amino termini of MRTFs contain Arg-Pro-X-X-X-Glu-Leu (RPEL) motifs, which form a stable complex with monomeric G-actin, resulting in the sequestration of MRTFs in the cytoplasm^{33,35-38}. Thus, mechanical forces promote actin assembly by phosphorylation of cofilin through Rho/Rho-kinase and LIMK, and force-induced actin assembly enhances the translocation of MRTF from the cytoplasm into the nucleus, and promotes transcription through SRF activation (Fig. 2)³⁰.

The actin-MRTF-SRF pathway plays an important role in force- and mechanosensing in cardiovascular systems. Cardiac hypertrophy, defined as the increase in the volume of the heart, is generally divided into physiological and pathological³⁹. Exercise-induced cardiac hypertrophy is typical physiological heart growth, and is accompanied by normal cardiac function⁴⁰. Pathological hypertrophy, on the other hand, is typically associated with the loss of cardiomyocytes (through both apoptosis and necrosis), fibrotic replacement, cardiac dysfunction, and increased risk of heart failure and sudden death^{40,41}. Pressure overload induces the rapid association between RhoA and Rho-kinase, and the activation of Rho-kinase in the adult rat myocardium⁴². Although both ROCK1 and ROCK2 are expressed in the myocardium, pressure overload only induces ROCK1 activation⁴³. Rho-kinase activity is also involved in the pathogenesis of left ventricular remodeling after myocardial infarction⁴⁴. RhoA is not required for normal homeostasis or for the initial compensatory hypertrophic response to stress⁴⁵. RhoA, however, regulates cardio-deleterious effects (fibrosis)⁴⁵. Chronic pathological stimulation leads to pathological hypertrophy associated with myoblast differentiation, a process in which cardiac fibroblasts express smooth muscle α -actin (SMA)⁴⁶. Mechanical stimulation enables the association of MRTF with SRF to induce the transcriptional activation of genes that induce fibrosis⁴⁶. Mechanical forces enhance actin assembly through the Rho/Rho-kinase-LIMK-cofilin pathway, which promotes the nuclear translocation of MRTF and SMA expression. mDia also regulates the expression of SMA in response to the translocation of MRTF induced by mechanical stimuli³¹.

Cyclic mechanical stretching also activates RhoA in skeletal muscles⁴⁷. MRTFs are required in skeletal muscle

Fig. 2 Rho/Rho-kinase controls localization of MRTF through actin polymerization

Actin filament formation alters the localization of the transcriptional co-activator MRTF (myocardin-related transcription factor), which, along with the transcription factor SRF (serum response factor), induces the expression of several target genes. The translocation of MRTF between the nucleus and the cytoplasm alters the transcription of genes regulating cardiac fibrosis and myogenic differentiation. Additionally, the nuclear localization of MRTF suppresses the expression of PPAR γ , a master regulator of adipogenesis. MRTF interacts with monomeric G-actin and is retained in the cytoplasm. Rho/Rho-kinase facilitates F-actin formation and decreases the concentration of G-actin through LIMK and cofilin. Thereby, Rho/Rho-kinase leads to the enhanced translocation of MRTF from the cytoplasm to the nucleus, and MRTF-SRF-dependent gene expression.

differentiation¹⁶⁾. In skeletal muscles, $\beta 1$ integrin is associated with RhoA activation in response to mechanical stress. Studies have shown that the dominant negative form of RhoA inhibits myogenic differentiation, while the constitutively active form of RhoA enhances myocyte growth and differentiation^{48,49)}. Mechanical overload enhances the expression of RhoA in the rat plantaris muscle⁵⁰⁾. On the other hand, tenotomy-induced disuse decreases RhoA activity in rodent gastrocnemius muscles⁵¹⁾. RhoA regulates the expression of the key skeletal muscle differentiation proteins: MyoD, and myogenin^{49,52)}. RhoA-dependent activation of SRF is mediated by MRTF-A and MRTF-B^{33,53)}. Rho/Rho-kinase signaling induces the expression of MyoD in an MRTF-SRF-dependent manner⁵⁴⁾.

Furthermore, cyclic mechanical stretching reduces the expression of the peroxisome proliferator-activated receptor gamma (PPAR γ), which is a master transcriptional regulator of adipogenesis, and inhibits the differentiation of mouse 3T3-L1 cells into adipocytes⁵⁵⁾. Depolymerization of the cytoskeleton also results in differentiation toward the adipogenic lineages⁵⁶⁾. RhoA and Rho-kinase play an important role in human mesenchymal stem cell (MSC) lineage commitment along the adipogenic pathway⁵⁷⁾. Latrunculin A (LatA) is an actin-depolymerizing reagent that increases monomeric G-actin levels⁵⁸⁾. Swinholide A (SwinA) is also an actin-depolymerizing reagent; however, it increases dimeric, and not monomeric, actin, which does not interact with MRTF-A^{33,58,59)}. LatA and SwinA

disrupt actin stress fibers and increase the concentration of G-actin monomers and dimers, respectively. Treatment with LatA induced a significant increase in cytoplasmic MRTF-A, and the expression of PPAR γ and PPAR γ -dependent genes⁵⁶. However, treatment with SwinA did not lead to the cytoplasmic localization of MRTF-A or the expression of PPAR γ and PPAR γ -dependent genes⁵⁶. These results suggest that monomeric G-actin formation leads to adipocyte differentiation by blocking the nuclear import of MRTF-A. Additionally, treatment with Y-27632, a Rho-kinase inhibitor, elicited the disruption of actin stress fibers and the cytoplasmic sequestration of MRTF-A, resulting in a marked increase in the expression of PPAR γ and PPAR γ -target genes, as well as in the accumulation of lipid droplets in cells⁵⁶. These results suggest that adipocyte differentiation is induced by the disruption of actin stress fibers through the suppression of the Rho/Rho-kinase signaling pathway⁵⁶. The depolymerization of actin induces the translocation of MRTF-A from the nucleus to the cytoplasm, and drives adipocyte differentiation through the transcriptional regulation of PPAR γ ⁵⁶. Thus, Rho/Rho-kinase signaling suppresses adipogenesis through the regulation of PPAR γ gene expression.

Rho/Rho-kinase regulates gene expression through YAP/TAZ in response to mechanical stimuli

It has been shown that cellular shape and stiffness of the extracellular matrix (ECM) influence the cell's decision to proliferate or differentiate⁶⁰. However, the mechanism underlying the regulation of cellular fate by cell shape and ECM stiffness is still unclear. Recently, it has been reported that mechanical signals are transduced to the nucleus by the transcriptional co-activators YAP and TAZ³². YAP and TAZ shuttle between the cytoplasm and the nucleus, where they interact with several promoter-specific transcription factors⁶¹. The TEA domain family member (TEAD) transcriptional factors appear to be the main partners of YAP and TAZ on DNA⁶¹.

The Hippo cascade has been characterized as a major regulator of YAP/TAZ. The Hippo pathway associates with mammalian STEM20-like protein kinase 1 and 2 (MST1/2, also known as STK4/3) and large tumor suppressor homologue 1 and 2 (LATS1/2)⁶¹. Activated MST1/2 phosphorylates LATS1/2, which in turn phosphorylates YAP/TAZ (Fig. 3)⁶¹. Phosphorylated YAP/TAZ are excluded from the nucleus and accumulate in the cytoplasm, where they are degraded by the protease or entrapped by 14-3-3 (Fig. 3)⁶⁰. On the other hand, when the Hippo pathway is inactivated, YAP/TAZ are dephosphorylated and translocate to the nucleus where they regulate gene transcription together with DNA-binding transcriptional factors such as TEADs⁶¹.

It has been reported that ECM stiffness and stretching induce YAP/TAZ activation and nuclear accumulation^{32,62}. ECM affects the localization of YAP/TAZ; they

are activated and localized to the nucleus in a rigid matrix whereas they are inactivated in an elastic matrix³². The inhibitor of actin polymerization, LatA, as well as, the Rho inhibitor, C3, and the Rho-kinase inhibitor, Y-27632, suppress the nuclear accumulation of YAP/TAZ³². These results indicate that the nuclear accumulation of YAP/TAZ is regulated by Rho/Rho-kinase signaling and actin structure³². However, knockdown of *LATS1* and *LATS2* was not sufficient to restore YAP/TAZ activity in the presence of LatA or in cells cultured in soft ECM hydrogels³². These results suggest that mechanical forces induce the translocation of YAP/TAZ, which is required for Rho/Rho-kinase signaling and the reorganization of actomyosin cytoskeleton, but are independent of the Hippo/LATS cascade³². The angiomin (AMOT) family has been identified as a YAP/TAZ- and F-actin-binding protein⁶³⁻⁶⁶. F-actin/AMOT and YAP/AMOT complexes may be complementary in function, such that the loss of F-actin may promote AMOT association with YAP^{67,68}. Actin polymerization may lead to the dissociation of YAP from AMOT, contributing to the nuclear translocation of YAP and YAP-dependent transcription.

YAP/TAZ are required for the differentiation of MSCs induced by ECM stiffness and survival of endothelial cells regulated by cell geometry³². MSCs differentiate into osteoblasts when seeded on a synthetic matrix engineered to have a bone-like stiffness, into myoblasts when grown on ECMs with intermediate stiffness, and into adipocytes when cultured on a soft ECM^{32,69}. RhoA and Rho-kinase activate YAP/TAZ, which enhances osteogenesis and suppresses adipogenesis, through actin polymerization³². Additionally, the nuclear localization of YAP/TAZ is suppressed by inhibition of non-muscle myosin. These results suggest that the nuclear translocation of YAP/TAZ is required not only for actin polymerization, but also for actomyosin contractility³². The depletion of YAP and TAZ inhibits osteogenic differentiation of MSCs while promoting adipocyte differentiation³². Therefore, Rho/Rho-kinase signaling regulates YAP/TAZ nuclear accumulation, which is required for differentiation of MSCs induced by ECM stiffness.

Conclusions

Previous studies have focused on the chemical and enzymatic signal transduction pathways that regulate cellular fate and function. However, it has previously been shown that cellular behavior is not only regulated by chemical and enzymatic factors; but tissue architecture and mechanical forces also influence cellular differentiation. Recently, MRTF and YAP/TAZ were identified as mechanical sensing transcriptional co-activators, a finding that provides a breakthrough in clarifying the molecular mechanisms of mechanotransduction. Mechanical stimuli induce the translocation of MRTF and YAP/TAZ from the cytoplasm to the nucleus by actin remodeling,

Fig. 3 Rho/Rho-kinase regulates transcription through YAP/TAZ in actin dynamics-dependent manner

Extracellular forces induce YAP/TAZ nuclear accumulation and gene expression changes. The activation of YAP/TAZ is regulated by Hippo-dependent and -independent pathways. Hippo-dependent pathways regulate YAP/TAZ activity by their phosphorylation through MST1/2 and LATS1/2. Phosphorylated YAP/TAZ is maintained in the cytoplasm by 14-3-3, or is degraded. On the other hand, mechanical stimuli regulate YAP/TAZ via Hippo-independent pathways. Mechanical stress activates RhoA/Rho-kinase signaling pathways, which enhances actin polymerization through LIMK and cofilin. F-actin formation enhances the nuclear accumulation of YAP/TAZ, whereas monomeric G-actin maintains the localization of YAP/TAZ in the cytoplasm. Namely, mechanical forces enhance gene expression thorough YAP/TAZ translocation induced by Rho/Rho-kinase-dependent actin polymerization.

which regulates the expression of several genes. Rho/Rho-kinase has been described as a key regulator of actin polymerization. Mechanical stress induces the activation of Rho/Rho-kinase signaling. Therefore, mechanical force regulates the transcriptional co-activators MRTF and YAP/TAZ through actin remodeling in response to the Rho/Rho-kinase signaling pathway. Rho/Rho-kinase-dependent gene expression changes regulate fibrosis,

osteogenesis, myogenesis, and adipogenesis. However, whether mechanical stimuli induce physiological and pathological biological responses has not yet been fully elucidated. Further studies are required to elucidate the molecular mechanisms underlying the regulation of the physiological and pathological effects in response to mechanical stress by Rho/Rho-kinase.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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