Rho Family Small G Proteins Play Critical Roles in Mechanical Stress–Induced Hypertrophic Responses in Cardiac Myocytes

Ryuichi Aikawa, Issei Komuro, Tsutomu Yamazaki, Yunzeng Zou, Sumiyo Kudoh, Weidong Zhu, Takashi Kadowaki, Yoshio Yazaki

Abstract—Mechanical stress induces a variety of hypertrophic responses, such as activation of protein kinases, reprogramming of gene expression, and an increase in protein synthesis. In the present study, to elucidate how mechanical stress induces such events, we examined the role of Rho family small GTP-binding proteins (G proteins) in mechanical stress–induced cardiac hypertrophy. Treatment of neonatal rat cardiomyocytes with the C3 exoenzyme, which abrogates Rho functions, suppressed stretch-induced activation of extracellular signal–regulated protein kinases (ERKs). Overexpression of the Rho GDP dissociation inhibitor (Rho-GDI), dominant-negative mutants of RhoA (DNRhoA), or DNRac1 significantly inhibited stretch-induced activation of transfected ERK2. Overexpression of constitutively active mutants of RhoA slightly activated ERK2 in cardiac myocytes. Overexpression of C-terminal Src kinase, which inhibits functions of the Src family of tyrosine kinases, or overexpression of DNRas had no effect on stretch-induced activation of transfected ERK2. The promoter activity of skeletal a-actin and c-fos genes was increased by stretch, and these increases were completely inhibited by either cotransfection of Rho-GDI or pretreatment with C3 exoenzyme. Mechanical stretch increased phenylalanine incorporation into cardiac myocytes by ~1.5-fold compared with control, and this increase was also significantly suppressed by pretreatment with C3 exoenzyme. Overexpression of Rho-GDI or DNRhoA did not affect angiotensin II–induced activation of ERK. ERKs were activated by culture media conditioned by stretch of cardiomyocytes without any treatment, but not of cardiomyocytes with pretreatment by C3 exoenzyme. These results suggest that the Rho family of small G proteins plays critical roles in mechanical stress–induced hypertrophic responses. (Circ Res. 1999;84:458-466.)

Key Words: Rho ■ mechanical stress ■ cardiac hypertrophy

Clinical studies have demonstrated that cardiac hypertrophy is not only an adaptational state before cardiac failure but also an independent risk factor for morbidity and mortality due to heart diseases.1 Thus, it has become even more important to understand the underlying mechanisms of the development of cardiac hypertrophy. Cardiac hypertrophy is often observed in patients who have hemodynamic overload.1 We and others have developed in vitro systems in which mechanical stress can be imposed on cultured cardiac myocytes and have demonstrated that mechanical stress induces a variety of hypertrophic responses, such as activation of protein kinases,2–5 reprogramming of gene expression,6,7 and an increase in protein synthesis2,4 in cultured cardiac myocytes. It remains unclear, however, how mechanical stress induces such intracellular events.

Integrins are a family of transmembrane receptors that bind to extracellular matrix (ECM) proteins, such as fibronectin, collagen, and vitronectin, and mediate a variety of cell functions.8–10 Integrins are heterodimeric proteins composed of noncovalently associated α- and β-subunits.8 Binding to the ECM and clustering of integrins lead to the formation of focal adhesions, by which integrins connect the ECM to intracellular actin stress fibers.10 This “scaffolding” structure also contains a number of molecules involved in signal transduction.11,12 In the in vitro stretch system, we culture cardiac myocytes of neonatal rats on a silicone membrane that has been precoated with collagen.6 Under these culture condition, cardiac myocytes attach to collagen on the silicone membrane via integrins in adhesion plaques. Therefore, it is conceivable that integrins first receive the stretch stimulus and then convert it into the biochemical signals. Recent studies have demonstrated that the cytoplasmic protein kinases, including focal adhesion kinase (FAK), are potential mediators of integrin signaling.13,14 FAK has been predicted...
to play a critical role in initiating cytoplasmic signals from sites of focal adhesion.\textsuperscript{13,14} We have reported that stretch activates FAK in mesangial cells cultured on silicone dishes.\textsuperscript{13} In cardiac myocytes, however, we could not detect significant enhancement of FAK activity by mechanical stretch because of its high basal activity (I.K. et al, unpublished observations, 1998). Although it has been reported that RGD peptide does not inhibit stretch-induced c-fos gene expression in cardiac myocytes,\textsuperscript{15} integrins that cannot be inhibited by RGD peptide may be involved in mechanical stretch–induced hypertrophic responses in cardiac myocytes.

The Rho family of small GTP-binding proteins, consisting of Rho, Rac, and Cdc42 subfamilies, regulates many aspects of cytoskeletal function.\textsuperscript{16} Rho has been shown to participate in the formation of focal adhesions and actin stress fibers, as well as in mediating the redistribution of cytoskeletal components.\textsuperscript{16,17} There is also strong evidence indicating that Rho plays a pivotal role in integrin-mediated signaling events. First, activated Rho stimulates stress fiber formation,\textsuperscript{18} and plating of cells onto fibronectin-coated dishes in serum-free medium results in the rapid formation of stress fibers.\textsuperscript{19} Second, both Rho and adhesion to fibronectin activate phosphatidylinositol-4-phosphate 5-kinase.\textsuperscript{19} Third, specific inhibition of Rho by the Clostridium botulinum C3 exoenzyme blocks certain cellular responses, an event that is similar to the loss of integrin-mediated cell adhesion.\textsuperscript{19} Last, overexpression of constitutively active Rho restores the ability of suspended cells to respond to growth factors.\textsuperscript{19}

Recently, integrin ligation has been demonstrated to induce activation and translocation to the nucleus of extracellular-regulated kinases (ERKs).\textsuperscript{20} In addition, Rho proteins are required for the activation of ERKs in NIH 3T3 cells plated on fibronectin.\textsuperscript{21} Although the molecular mechanism by which Rho family proteins regulate integrins still remains to be determined, all of these observations suggest that Rho is critically involved in integrin-mediated intracellular signaling.

In the present study, we examined the role of Rho family small G proteins in the development of mechanical stress–induced hypertrophic responses. We demonstrate that Rho family proteins play important roles in stretch-induced activation of ERKs, expression of skeletal \(\alpha\)-actin and c-fos genes, and an increase in protein synthesis in cardiac myocytes.

### Materials and Methods

\[\text{[y-32P]ATP was purchased from Du Pont–New England Nuclear Co. Dulbecco’s modified Eagle’s medium (DMEM), FBS, and genistein were from Gibco BRL Co. An anti-ERK (\(\alpha\)Y91) antibody was provided by K. Tobe (University of Tokyo Graduate School of Medicine, Tokyo, Japan). C3 exoenzyme was a kind gift from S. Narumiya (Kyoto University, Graduate School of Medicine, Kyoto, Japan). An anti-hemagglutinin (HA) polyclonal antibody was from MBL, Nagoya, Japan. Angiotensin (Ang) II, insulin, myelin basic protein (MBP), and other reagents were purchased from Sigma Chemical Co.}\]

### cDNA Plasmids

HA-tagged ERK2 (HA-ERK2) and the dominant-negative mutant (Asn-17) of Ras (DNRas) were kind gifts from M. Karin (University of California, San Diego, Calif)\textsuperscript{24} and Y. Takai (Osaka University Faculty of Medicine, Osaka, Japan). Rho-GDI and various mutants of RhoA, Rac1, and Cdc42 were from J.S. Gutkind (National Institute of Dental Research, Bethesda, Md),\textsuperscript{26} and wild-type C-terminal Src kinase (CSK) and the kinase-negative mutant of CSK (CSK) were kindly provided by H. Sabe (Kyoto University, Kyoto, Japan). The dominant-negative mutant (Ala-17) of Raf-1 (DNraf-1) was described previously.\textsuperscript{28} All plasmid DNA was prepared by using QIAGEN plasmid DNA preparation kits.

### Cell Culture

Primary cultures of cardiac myocytes were prepared from ventricles of 1-day-old Wistar rats as described previously\textsuperscript{6} according to the method of Simpson.\textsuperscript{29} In brief, cardiomyocytes were plated at a field density of \(10^5\) cells/cm\(^2\) on silicone dishes with 2 mL of culture medium (DMEM with 10% FBS).\textsuperscript{6} Twenty-four hours after seeding, the culture medium was changed to a solution of serum-free DMEM for 48 hours before treatment.

### Transfection

Twenty-four hours after plating the cells on culture dishes, we transfected DNA by using the calcium phosphate method as described previously.\textsuperscript{6} For each dish, 2 \(\mu\)g of HA-ERK2 plasmid DNA was transfected with 6 \(\mu\)g of the other relevant plasmids, such as DNraf-1, CSK, or empty vector. After 15 hours of transfection, the culture medium was removed, and the cells were washed twice with PBS and maintained in serum-free DMEM for 48 hours before stretching or treatment with chemical reagents. For the luciferase assay, 2 \(\mu\)g of c-fos or skeletal \(\alpha\)-actin promoter luciferase plasmid was transfected into cardiomyocytes with Rho-GDI or empty vector by using the calcium phosphate method. After 6 or 12 hours of transfection, the culture medium was removed, and cells were washed twice with PBS and maintained in serum-free DMEM for 48 hours before stretching or treatment with chemical reagents.

### Kinase Assay of Endogenous ERKs

Endogenous ERK activity was measured by using MBP as a substrate.\textsuperscript{2,22} In brief, cardiomyocytes were lysed with buffer A (25 mmol/L Tris-HCl, pH 7.4; 25 mmol/L NaCl; 1 mmol/L sodium orthovanadate; 10 mmol/L sodium pyrophosphate; 10 mmol/L oka-
diac acid; 0.5 mmol/L EGTA; and 1 mmol/L PMSF), and the lysates were incubated with an anti-ERK polyclonal antibody for 1 hour at 4°C. After incubation, the immunocomplex was precipitated by using protein A–Sepharose beads, washed, and resuspended, and incubated again with 25 \(\mu\)mol/L ATP at 25°C for 10 minutes in 25 \(\mu\)L of kinase buffer (25 mmol/L Tris-HCl, pH 7.4; 10 mmol/L MgCl\(_2\); 1 mmol/L DTT; 40 \(\mu\)mol/L ATP; 2 \(\mu\)Ci [\(\gamma\)-32P]ATP; 2 \(\mu\)mol/L protein kinase inhibitor peptide; and 0.5 mmol/L EGTA). After incubation, the reaction was terminated by adding Laemmli sample buffer (0.002% bromophenol blue, 0.01 mmol/L sodium phosphate buffer [pH 7.0], 10% glycerol, 0.4% SDS, and 1% 2-mercaptopethanol) to the samples, and the samples were boiled for 5 minutes. The supernatants were subjected to SDS–polyacrylamide gel electrophoresis, and then the gel was washed with 7% acetic acid for 30 minutes and with 3% glycerol for 30 minutes, dried, and subjected to autoradiography.

### Kinase Assay of Transfected HA-ERK2

After stimulation, cardiomyocytes into which HA-ERK2 was transfected were lysed with lysis buffer A, and the lysates were incubated with anti-HA polyclonal antibody for 1 hour at 4°C. After incubation, the immunocomplex was precipitated by using protein A–Sepharose beads, washed, and resuspended, and incubated again with 25 \(\mu\)mol/L MBP at 25°C for 10 minutes in 25 \(\mu\)L of the kinase buffer, and incubated with 25 \(\mu\)mol/L MBP as a substrate at 25°C for 10 minutes. After incubation, the reaction was terminated by adding the Laemmli sample buffer to the samples. The supernatants were subjected to SDS–polyacrylamide gel electrophoresis, and the gel was dried and subjected to autoradiography.
Mechanical stress was im-
ribosylates small G proteins of the Rho subfamily at Asn-41, 
cardiac myocytes. C3 exoenzyme selectively ADP-
exoenzyme on mechanical stress–induced ERK activation in 

After starvation for 48 hours, cardiac myocytes were stretched by 
20% for 24 hours. The relative amount of protein synthesis was 
determined by assessing the incorporation of radioactivity into a 
trichloroacetic acid–insoluble fraction as previously reported.3,4 

C3 exoenzyme inhibits stretch-induced activation of 
ERKs in cardiomyocytes. Cultured cardiac myocytes were 
stretched by 20% for 9 minutes. Some cardiomyocytes were 
pretreated with 10 μg/mL C3 exoenzyme for 36 hours (C3 + Stretch). Treatment with C3 exoenzyme had no effect on 
the activity of ERKs (C3). ERKs were immunoprecipitated from 
cell lysates by using a polyclonal antibody against ERKs (αY91), 
and kinase activity was assayed by using MBP as a substrate. 
After electrophoresis, the gel was dried and subjected to auto-
radiography. A representative autoradiogram from 3 independ-
ent experiments is shown.

It has also been reported that Rho proteins are involved in the 
integrin-dependent activation of ERKs21 and that Rho pro-

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ERKs, as described previously.2,3 Although pre-
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activity, the increase in ERK activity induced by stretch was 
strongly (~90%) inhibited by treatment with the C3 exoen-
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We further examined the role of Rho proteins in stretch-
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Results
Rho Is Important for Stretch-Induced Activation of 
ERKs in Cardiac Myocytes

Rho family members such as RhoA, Rac1, and Cdc42 have 
been reported to regulate the activity of mitogen-activated 
protein kinase (MAPK) subfamilies such as c-Jun NH2-
terminal kinase and p38 MAPK in HeLa and COS-7 cells.26,30 
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The Src Family of Tyrosine Kinases or Ras Is Not Involved in Stretch-Induced Activation of ERKs in Cardiomyocytes

Stimulation of receptor tyrosine kinases activates the Raf-1-MAPK/ERK kinase (MEK)-ERK cascade through Ras in many cell types. Activation of the Src family of tyrosine kinases and Ras has also been reported to be required for Ang II–induced activation of ERKs in smooth muscle cells. It has recently been reported that mechanical stress stimulates the secretion of Ang II from cardiac myocytes and that Ang II activates Src family tyrosine kinases and Ras in cardiac myocytes. We thus examined the role of the Src family of protein kinases and the small G protein Ras in stretch-induced ERK activation by overexpressing C-terminal Src kinase (CSK) and DNRas, respectively. CSK has been reported to phosphorylate and inactivate Src family protein kinases. We transfected HA-ERK2 into cardiomyocytes with a kinase-negative mutant of CSK (CSK), wild-type CSK (CSK), or DNRas and stretched the cardiomyocytes by 20% for 9 minutes. Stretch-induced ERK activation was not attenuated by overexpression of CSK or DNRas (Figure 4A). On the other hand, activation of the transfected ERK2 by stretch was completely suppressed by cotransfection of DNRaf-1 (Figure 4A), indicating that stretch activates ERKs via a pathway involving Raf-1 kinase but not Src family protein kinases or Ras proteins in cardiac myocytes. By contrast, the insulin-induced increase in ERK2 activity was significantly inhibited by cotransfection of CSK but not by CSK and completely suppressed by cotransfection of DNRas and DNRaf-1 (Figure 4B).

Rho Is Required for Stretch-Induced Expression of c-fos and Skeletal α-Actin Genes in Cardiac Myocytes

Expression of immediate-early response genes and fetal genes is a genetic response of cardiac myocytes to mechanical stress. We examined the role of Rho proteins in mechanical stress–induced gene expression in cardiac myocytes. A reporter gene containing the c-fos gene promoter was transfected into cardiac myocytes. The luciferase activity of the c-fos reporter gene was increased by stretch, and this increase was completely inhibited by pretreatment with the C3 exoenzyme (Figure 5A). When Rho-GDI was transfected with the c-fos reporter gene into cardiac myocytes, mechanical stress did not increase reporter gene luciferase activity (Figure 5A). Next, we examined the role of Rho proteins in stretch-induced fetal gene expression in cardiac myocytes. Mechanical stress–induced activation of skeletal α-actin promoter was also markedly suppressed by pretreatment with the C3 exoenzyme or overexpression of Rho-GDI (Figure 5B). These results suggest that Rho proteins play a critical role in the mechanical stress–induced reprogramming of gene expression.

Rho Is Required for the Stretch-Induced Increase in Phenylalanine Incorporation Into Cardiomyocytes

To determine whether Rho proteins are involved in stretch-induced cardiac hypertrophy, we examined relative protein synthesis after stretch in the presence or absence of the C3 exoenzyme. Stretch by 20% for 24 hours increased phenylalanine incorporation into cardiac myocytes by ~1.5-fold compared with controls (Figure 6). This increase was significantly (~70%) suppressed by pretreatment with 10 μg/mL of the C3 exoenzyme.
for 36 hours, suggesting that Rho proteins play a critical role in the mechanical stress–induced cardiac hypertrophy.

Rho Proteins Are Not Involved in Ang II–Induced ERK Activation in Cardiomyocytes

We have reported that Ang II partly mediates the development of mechanical stress–induced hypertrophic responses.2,3 Therefore, we examined the relationship between Ang II and Rho proteins. We first asked whether Rho proteins are involved in Ang II–induced ERK activation in cardiomyocytes. The transfected ERK2 was activated by 10^{-6} mol/L Ang II as reported previously,40 but activation of ERK2 was not attenuated by pretreatment with the C3 exoenzyme (Figure 7). In addition, overexpression of Rho-GDI and DNRhoA also did not have any effect on Ang II–induced ERK activation (Figure 7). Next, we investigated whether Rho proteins affect Ang II–induced ERK activation during stretching. The activation of transfected ERK2 induced by stretch was significantly (~60%) inhibited by the Ang II type 1 receptor–specific antagonist CV-11974 (10^{-6} mol/L), as reported before (Figure 8). When cardiomyocytes were transfected by Rho-GDI and pretreated with CV-11974, activation of transfected ERK2 by stretch was more strongly suppressed (Figure 8). These results suggest that Rho proteins are not involved in the process of Ang II–induced activation of ERKs during mechanical stress–induced cardiac hypertrophy.

Figure 6. Rho is required for the stretch-induced increase in protein synthesis. After pretreatment with 10 μg/mL C3 exoenzyme for 36 hours, cardiac myocytes were stretched by 20% for 24 hours, and [3H]phenylalanine (1 μCi/mL) was added 2 hours before harvest. The total radioactivity of incorporated [3H]phenylalanine into proteins was determined by liquid scintillation counting. The data represent the mean percentage of controls (100%) from 3 independent experiments (mean±SE). *P<0.05 versus control and #P<0.05 versus stretch without any inhibition of Rho function.

Rho Proteins May Play an Important Role in Ang II Secretion

It has recently been reported that Ang II is secreted from stretched cardiomyocytes.41 We examined whether Rho proteins are involved in Ang II secretion by using the culture
media of cardiomyocytes. The culture media of cardiomyocytes that were not stretched or stretched by 20% for 10 minutes were transferred to cardiomyocytes of another culture dish, and activity of ERK was measured as previously reported.39 The addition of conditioned media by stretch significantly increased ERK activity (Figure 9). Pretreatment of recipient cells with CV-11974 (10^−6 mol/L) potently suppressed ERK activation induced by addition of the conditioned media, suggesting that Ang II in the stretch-conditioned media activated ERKs (Figure 9). When stretch-conditioned media were prepared from cells pretreated with the C3 exoenzyme, ERK activity was not increased (Figure 9). These results collectively suggest that Rho proteins may play a critical role in stretch-mediated ERK activation in cardiac myocytes.

**Discussion**

Mechanical stress induces reprogramming of gene expression and an increase in protein synthesis in cardiac myocytes.2,4–7 We and others have reported that mechanical stretch activates the Raf-1 kinase–MEK-ERK cascade partly through secreted Ang II and endothelin-1.3,5,40,42 Moreover, c-Jun NH2-terminal kinases, a subfamily of MAPKs, are also activated by mechanical stress and Ang II in cardiomyocytes.3,44 Although activation of the protein kinase cascade of phosphorylation has been reported to be important for the development of hypertrophic responses,3,5,42,43 it remains unknown how mechanical stress is converted into these biochemical signals. Interaction of cells with the ECM via integrins plays an important role in a wide range of cellular functions, such as cell growth, movement, and differentiation.8 After cell adhesion to ECM components, many intracellular events, including phosphorylation of FAK and FAK-associated tyrosine kinase, occur.13,14 Integrin-mediated formation of focal adhesions and phosphorylation of FAK need an intact cytoskeleton.13,14 A growing body of evidence has suggested that Rho family proteins are critically involved in regulation of the cytoskeleton, such as formation of actin microfilaments and focal adhesions.18,45 Integrins have been reported to activate ERKs when bound to the ECM,11,12,21 and Rho proteins are involved in the integrin-dependent activation of ERKs.21 Therefore, we examined whether the Rho family of small G proteins is involved in mechanical stress–induced ERK activation in cardiomyocytes plated on collagen-coated, silicone membranes. In the present study, mechanical stress–induced activation of ERKs was strongly suppressed by overexpression of Rho-GDI or pretreatment with the C3 exoenzyme in cardiac myocytes. These results suggest that Rho proteins are involved in ERK activation induced by mechanical stress. In addition, overexpression of CARhOA or CARac1 had marginal effects on ERK in unstretched cardiomyocytes, and these active mutants of the Rho family did not enhance the transfected ERK2 activity in stretched cardiomyocytes (Figure 3). These results suggest that Rho proteins may be required for mechanical stretch–induced hypertrophic responses by modulating the functions of integrins.

Hypertrophy of neonatal ventricular myocytes is associated with a number of phenotypic changes, including reprogramming of gene expression, changes in cell shape, and organization of contractile proteins into sarcomeric units.37,38,46 It has recently been reported that Rho is required for gene expression mediated by growth factors, including FGF-1.37,38,46
expression that is induced by signals through Gaq and α1-adrenergic receptors and that Rho-dependent pathways are involved in phenylephrine (PHE)-induced atrial natriuretic factor gene expression by interacting with Ras cascades in cardiac myocytes.\textsuperscript{47} In addition, the activation of ERKs through Ras is required for PHE-induced gene expression, and Ras but not Rho plays a critical role in actin organization in cardiac myocytes.\textsuperscript{48,49} We showed that Rho proteins are essential for mechanical stress–induced expressions of c-fos and skeletal α-actin genes (Figure 5A and 5B). These results suggest that Rho plays a critical role in the stretch-induced reprogramming of gene expression as well as in PHE-induced gene expression. Although inhibition of Rho protein functions partly inhibited stretch-induced activation of ERKs, stretch-induced gene expression was mostly suppressed by pretreatment with the C3 exoenzyme and overexpression of Rho-GDI. These results suggest that the role of Rho proteins in gene expression may be different from their role in ERK activation. Recent studies have demonstrated that Rho proteins are involved in transcriptional activation of the c-fos gene by regulating serum response factor (SRF) in several cell types, including cardiac myocytes, and activation of the SRF-linked signaling pathway is not correlated with the activation of ERKs.\textsuperscript{50,51} Moreover, because the CArG sequence, a regulatory sequence found in many muscle-specific promoters including the skeletal α-actin gene promoter,\textsuperscript{52} is similar to the c-fos serum response element, the skeletal α-actin gene promoter as well as the c-fos gene promoter may be directly regulated by Rho proteins via CArG/serum response element sequences in cardiac myocytes.

Recently, we have demonstrated that mechanical stress activates ERKs partly through secreted Ang II in cardiomyocytes.\textsuperscript{59} In the present study, however, Rho proteins had little effect on the activation of ERK2 induced by Ang II (Figure 7), as in the case of PHE.\textsuperscript{47,49} Stretch-induced ERK activation was more strongly suppressed by combined treatment, ie, overexpression of Rho-GDI and treatment with an Ang II type I receptor antagonist, compared with single treatment (Figure 8). These results suggest that Rho proteins are not involved in the process of Ang II–induced ERK activation during mechanical stress–induced cardiac hypertrophy. As shown in Figure 9, pretreatment of cardiomyocytes with the C3 exoenzyme potently suppressed Ang II secretion from stretched cardiomyocytes. Recent studies have indicated that Rho is involved in the release of humoral factors such as catecholamines and gastrin in some cell types.\textsuperscript{33,54} Taken together, although Rho proteins do not mediate Ang II–induced hypertrophic responses, it is conceivable that Rho proteins regulate the secretion of Ang II in stretched cardiomyocytes.

The small G protein Ras plays a key role in a variety of cell functions partly through Raf-1 and ERKs.\textsuperscript{55,56} We have recently reported that oxidative stress activates ERKs through Ras in cardiac myocytes.\textsuperscript{57} In addition, it has been reported that hypertrophy-promoting stimuli, such as PHE and Ang II, activate Ras in cardiac myocytes.\textsuperscript{35,58} Ras is usually activated by tyrosine kinases, including the Src family of tyrosine kinases,\textsuperscript{55,59} and it has also been reported that Ang II activates Ras through Fyn, 1 of the Src family tyrosine kinases in cardiac myocytes.\textsuperscript{35} We therefore examined whether Ras and Src family tyrosine kinases were required for stretch-induced activation of ERKs in cardiomyocytes. Although insulin-induced ERK activation was suppressed by overexpression of DNRas and CSK (Figure 4B), DNRas or CSK had no effect on stretch-induced activation of ERKs (Figure 4A). These results suggest that although the small G protein Ras and Src family tyrosine kinases may be activated by mechanical stretch, they do not play a major role in mechanical stress–induced activation of ERKs.

The Rho family of small G proteins plays a critical role in mechanical stress–induced hypertrophic responses, such as activation of ERKs, expression of specific genes, and an increase in protein synthesis. Mechanical stretch activates ERKs, possibly through Ang II–dependent and –independent pathways (Figure 10). Because inhibition of Rho family functions had strong effects on stretch-evoked signal transduction, the Rho family may be involved in both pathways. The almost-complete inhibition of stretch-induced gene expression may indicate another role of Rho proteins. Recent studies have demonstrated that Rho proteins regulate c-fos gene expression through an SRF-linked signaling pathway.\textsuperscript{50,51} Recently, several studies have also demonstrated that Rho proteins are regulated by the integrity of the cell wall in yeast.\textsuperscript{60,61} When cell wall integrity is changed during growth at high temperature, the MAPK cascade is activated through Rho1, a yeast homologue of mammalian RhoA, in yeast cells.\textsuperscript{60,61} Therefore, the phenomenon that mechanical stress activates the MAPK family through Rho small G proteins may be conserved in evolution. Further investigation is required to elucidate how Rho proteins are involved in the regulation of stretch-induced cardiac hypertrophy.

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References


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