Rho-ROCK-LIMK-Cofilin Pathway Regulates Shear Stress Activation of Sterol Regulatory Element Binding Proteins

Tong Lin,* Lingfang Zeng,* Yi Liu, Kathryn DeFea, Martin A. Schwartz, Shu Chien, John Y.-J. Shyy

Abstract—Previous studies have shown that integrin activation and fluid shear stress can modulate the activity of sterol regulatory element binding proteins (SREBPs) in vascular endothelial cells. We investigated the role of small GTPase Rho-mediated signal transduction pathway in this mode of SREBP activation. Fluid shear stress activates the Rho downstream effectors ROCK, LIM kinase (LIMK), and cofilin. The various negative mutants of RhoA, ROCK, LIMK, and cofilin can block the shear stress activation of SREBPs. The shear stress–activated SREBP depends on S2P proteases but not caspase-3. Mechanistically, the endoplasmic reticulum-to-Golgi transport of SREBP cleavage–activating protein requires the actin-based cytoskeleton and is enhanced by the Rho-ROCK-LIMK-cofilin pathway. By enhancing the SREBP-mediated cholesterol metabolism, this unique mechanism may contribute to endothelial cell functions under flow. (Circ Res. 2003;92:1296-1304.)

Key Words: mechanotransduction  endothelial cells  sterol regulatory element binding proteins  shear stress  Rho

Sterol regulatory element binding proteins (SREBPs), including SREBP1a, SREBP1c, and SREBP2, are key regulators of cellular sterol and lipid homeostasis (see review).1 SREBP1a and SREBP2 preferentially regulate genes involved in cholesterol metabolism, whereas SREBP1c modulates genes engaged in fatty acid synthesis.2,3 In response to sterol depletion, SREBPs are transported from the endoplasmic reticulum (ER) to the Golgi by SREBP cleavage–activating proteins (SCAPs), which results in 2 proteolytic cleavages at the Golgi by S1P and S2P proteases.4–6 In addition to being regulated by the cellular content of sterols, SREBP cleavage can be induced by inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6.7,8 and epidermal growth factor (EGF).9 It has been suggested that the SREBP1 cleavage by TNF-α is catalyzed by caspase-3 (ie, CPP32) through the sphingomyelinase (N-SMase)-ceramide pathway.10,11

By switching between a GDP-bound inactive and a GTP-bound active form, Rho can regulate the cytoskeletal structure, gene transcription, specific cell fate, and cell transformation.12,13 Rho is directly linked to integrin-mediated signaling.14 ROCK, a Rho-associated serine/threonine kinase, has been characterized as an effector of Rho.15 The expression of kinase-defective mutants of ROCK in cells causes the disassembly of stress fibers and focal adhesions.16 In contrast, constitutively active forms of Rho or ROCK induce stress fiber formation.17 Cofilin is an actin-binding protein that is essential for the depolymerization of actin filaments.18,19 By inducing cofilin phosphorylation, Rho abolishes the actin-binding activity of cofilin, thereby enhancing the polymerization of actin filaments.20 LIM kinase (LIMK) can regulate actin dynamics through the phosphorylation of cofilin.21–24 Thus, Rho regulates cofilin via ROCK and LIMK, and this signal transduction pathway modulates actin assembly in many cell types in response to various extracellular stimuli.

Shear stress acting on the vascular endothelial cells (ECs) plays a crucial role in the homeostasis of the vascular wall.25 An increasing body of evidence suggests that the link among integrins, Rho small GTPase, and the actin-based cytoskeleton is important in mechanotransduction elicited by shear stress.26 We have shown that shear stress activates SREBPs in ECs, with a concurrent modulation of genes regulated by SREs such as low-density lipoprotein receptor (LDLR) and fatty acid synthase.27 Interestingly, this regulation depends on integrin function and actin integrity. The involvement of integrin in this SREBP activation is additionally supported by the observation that cell attachment to the extracellular matrix (ECM) also activates SREBP1.27 In this study, we provide evidence that shear stress activates the Rho-ROCK-LIMK-cofilin pathway, which in turn modulates the activation of SREBP.

Materials and Methods

Cell Cultures and Shear Stress Experiments

Bovine aortic ECs (BAECs) of passages 5 through 10 were maintained in DMEM supplemented with 10% FBS in a humidified 5%
CO₂ and 95% air incubator at 37°C. The wild-type Chinese hamster ovary cells (CHO-K1) were grown in medium A (a mixture of Ham’s F-12 medium and DMEM [1:1 vol/vol] containing 100 U/mL penicillin and 100 μg/mL streptomycin sulfate) supplemented with 5% FCS.

For shear stress experiments, BAECs or CHO cells were cultured on glass slides (38×76 mm) to confluence. A silicone gasket was sandwiched between the glass slide and an acrylic plate to create a rectangular flow channel (0.025 cm high, 2.5 cm wide, and 5.0 cm long). Cells were exposed to the applied shear stress generated by circulating the tissue culture media through a hydrostatic pump connected to the upper and lower reservoirs. The pH of the system was kept constant by gassing with humidified 95% air and 5% CO₂, and the temperature was maintained at 37°C. The shear stress, determined by the flow rate perfusing the channel and the channel dimensions, was 12 dyne/cm². Static control experiments were performed on cells kept on slides without being exposed to shear stress for the same duration.

DNA Plasmids and Transient Transfection
To clone the hemagglutinin (HA)-tagged SREBP2 (HA-SREBP2), the 4.2-kb BspDI/XbaI fragment of human SREBP2, corresponding to amino acids 14 to 1141, was released from plasmid pTK-HSV-BP2 (ATCC) and subcloned into the BglII/XbaI site of pCMV5-Nter-HA-HA to create the HA-SREBP2 driven by a cytomegalovirus

Figure 1. Shear stress activates ROCK and LIMK. BAECs were either kept as static controls (represented by time 0) or subjected to shear stress for 1, 5, 15, 30, and 120 minutes. A, ROCK was immunoprecipitated from the cell lysates by a polyclonal anti-ROCK. MBP and [γ-32P]ATP were used for the ROCK activity assay. The autoradiographic bands represent the phosphorylated MBP. B, LIMK2 was immunoprecipitated from the cell lysates by a polyclonal anti-LIMK2 for kinase activity assay using cofilin and [γ-32P]ATP. Shown in the bottom panels of A and B are MBP or cofilin Coomassie blue staining, indicating comparable loadings.

Figure 2. Shear stress activates the Rho-ROCK-LIMK-cofilin pathway. A, BAECs were transfected with HA-LIMK2 (1 μg), together with 0.2, 0.5, or 1.0 μg Myc-RhoN19 and appropriate amounts of supplemented pcDNA3. Transfected cells were either kept as static controls (lanes 1 through 4) or subjected to shear stress for 30 minutes. HA-LIMK2 was immunoprecipitated from cell lysates by a polyclonal anti-HA for kinase activity assay using cofilin as substrates. Shown in the middle and bottom panels are immunoblotting results with anti-Myc or anti-HA, demonstrating the expression of the exogenous Myc-RhoN19 and HA-LIMK2. The procedures in B were the same as those in A except that BAECs were cotransfected with HA-LIMK2 (1 μg) together with 0.2, 0.5, or 1.0 μg Myc-ROCK-C. The middle and bottom panels of B are immunoblotting results with anti-Myc or anti-HA, demonstrating the expression of the exogenous Myc-ROCK-C and HA-LIMK2. Data are representative results from 3 separate experiments.
A total ROCK was immunoprecipitated from the cell lysates by polyclonal anti-ROCK (Santa Cruz Biotechnology, Santa Cruz, Calif). A kinase activity assay was performed with MBP as the substrate. The samples were then separated by 10% SDS-PAGE and underwent autoradiography to visualize the phosphorylated MBP. The procedure for the LIMK activity assay was the same as that for the ROCK assay except that polyclonal anti-LIMK2 (Santa Cruz Biotechnology) was used in immunoprecipitation and 10 μg of purified cofilin was used as the substrate in the kinase reaction.

**Immunostaining and Fluorescence Microscopy**

The effect of Rho on the nuclear translocation of SREBP2 in response to shear stress was investigated by immunostaining cells cotransfected with Myc-RhoN19 and HA-SREBP2. The transfected cells were washed with PBS, fixed with paraformaldehyde (3.7% in PBS) for 10 minutes, permeabilized with Triton X-100 (0.2% in PBS) for 5 minutes, and blocked with BSA (1% in PBS). The specimens were then incubated with mouse anti-HA mAb (1:50, vol/vol, Santa Cruz Biotechnology), rabbit polyclonal anti-Myc (1:50, vol/vol, Santa Cruz Biotechnology), and FITC-labeled phalloidin (1:200, Molecular Probe) in PBS containing 1% BSA for 1 hour. A PBS solution containing rhodamine-conjugated anti-mouse IgG (1:100, vol/vol, Jackson Immunoresearch), Cy5-conjugated anti-rabbit IgG (1:100, vol/vol, Jackson Immunoresearch), and 1% BSA was then applied to the cells, which were then incubated at reverse transcriptase for 30 minutes in the dark. The immunostaining results were observed under a Zeiss LSM 510 laser scanning confocal microscope. FITC was excited at a wavelength of 488 nm and detected between 506 and 538 nm, rhodamine was excited at 568 nm and detected between 589 and 621 nm, and Cy5 was excited at 650 nm and detected at 670 nm.

**Immunoblotting**

BAECs or CHO cells were lysed, and the protein samples were subjected to SDS-PAGE according to standard protocols. After the proteins in the gel were transferred to nitrocellulose membranes, the blot was blocked with 5% BSA in TTBS (10 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, and 0.05% Tween 20) followed by incubation with various primary antibodies in TTBS containing 1% BSA. The bound primary antibodies were detected with horseradish peroxidase–conjugated IgG and the ECL detection system (Amer- sham Pharmacia Biotech).

**Results**

**Shear Stress Activation of the Rho-ROCK-LIMK-Cofilin Pathway**

Shear stress causes the translocation of Rho from the cytosol to the cytoplasm membrane. To delineate the roles of Rho downstream effectors ROCK, LIMK, and cofilin in SREBP activation in response to shear stress, we first investigated whether the ROCK-LIMK-cofilin pathway is activated by shear stress. Confluent monolayers of BAECs were subjected to shear stress for times ranging from 1 minute to 2 hours. Equal amounts of cell lysates were immunoprecipitated with anti-ROCK, and kinase activity assays were performed with MBP as the substrate. Demonstrated by MBP phosphorylation, shear stress caused a rapid increase in the kinase activity of ROCK, which returned to the basal level at later times (Figure 1A). Phosphorylation of cofilin demonstrated that shear stress also activated LIMK2 in a more sustained manner (Figure 1B). We additionally explored whether Rho and ROCK

**Kinase Activity Assay**

ROCK was immunoprecipitated from the cell lysates by polyclonal anti-ROCK (Santa Cruz Biotechnology, Santa Cruz, Calif). A total of 30 μL of the kinase assay buffer with 25 μCi of [γ-32P]ATP, 25 μmol/L ATP, and 5 μg MBP was added to each immunocomplex pellet and incubated at 30°C for 30 minutes. The kinase reaction was terminated by adding 4× SDS sample buffer. The samples were then separated by 10% SDS-PAGE and underwent autoradiography to visualize the phosphorylated MBP. The procedure for the LIMK activity assay was the same as that for the ROCK assay except that polyclonal anti-LIMK2 (Santa Cruz Biotechnology) was used in immunoprecipitation and 10 μg of purified cofilin was used as the substrate in the kinase reaction.
regulate LIMK to increase the phosphorylation of cofilin in response to shear stress. HA-LIMK2 was cotransfected with increasing amounts of Myc-RhoN19 (Figure 2A), a dominant-negative mutant of RhoA, or Myc-ROCK-C (Figure 2B), which encodes the Rho-binding domain of ROCK and functions as a dominant-negative mutant of ROCK.\textsuperscript{15} The transfected cells were then either subjected to shear stress (lanes 5 through 8 in Figures 2A and 2B) or kept as static controls (lanes 1 through 4 in Figures 2A and 2B). The shear-induced cofilin phosphorylation mediated by HA-LIMK2 was attenuated by the expression of Myc-RhoN19 (Figure 2A) or Myc-ROCK-C (Figure 2B) in a dose-dependent manner (lane 5 versus 1, 6 versus 2, 7 versus 3, and 8 versus 4). The results presented in Figures 1 and 2 demonstrate that shear stress activates the Rho-ROCK-LIMK-cofilin pathway in ECs.

**ROCK, LIMK, and Cofilin Are Necessary for Rho-Activated SREBP2**

Because both cell attachment and shear stress modulate Rho activity and SREBP maturation,\textsuperscript{27,29} we investigated whether SREBP2 is downstream of Rho in response to shear stress. ECs were cotransfected with HA-SREBP2 and either pCDNA3 or Myc-RhoN19, followed by shear stress stimulation. Immunostaining revealed that shear stress caused a strong nuclear staining of HA-SREBP2 in cells cotransfected with pCDNA3 (Figure 3A). Under static conditions, the antigen recognized by anti-HA distributed mainly in the cytoplasm (Figure 3C, left). These results indicate the SREBP2 activation by shear stress. In contrast, cells transfected with Myc-RhoN19 had HA-SREBP2 staining exclusively outside the nucleus (Figure 3B). The shear stress-induced HA-SREBP2 nuclear translocation is similar to that
induced by a medium supplemented with lipoprotein-deficient serum (LDS), whereas the inclusion of 15% FBS had similar inhibitory effects as Myc-RhoN19 on HA-SREBP2 nuclear translocation (Figure 3C).

Having demonstrated that shear stress activated the Rho-ROCK-LIMK-cofilin pathway and that RhoN19 inhibited the shear stress–induced SREBP2 nuclear translocation, we investigated whether ROCK, LIMK, and cofilin are involved in the shear stress activation of SREBP2. BAECs were transfected with wild-type epitope-tagged ROCK (Myc-ROCK), LIMK2 (HA-LIMK2), or cofilin (Flag-cofilin), together with HA-SREBP2 or Flag-SREBP2. In parallel experiments, cells were transfected with their respective dominant-negative mutants [ie, Myc-ROCK-C, HA-LIMK2(KD), and Flag-cofilin(S3A)]. As shown in lanes 2 and 4 in Figures 4A through 4C, shear stress caused an increase in HA-SREBP2 cleavage in cells transfected with the wild-type ROCK, LIMK2, or cofilin (lane 6 versus lanes 2 and 4 in Figures 4A through 4C).

To confirm additionally that the ROCK-LIMK-cofilin pathway regulates the SREBP-mediated transcriptional activation, BAECs were cotransfected with the reporter construct 4SRE-Luc together with the respective dominant-negative mutants of ROCK, LIMK2, or cofilin and the empty plasmid pcDNA3. As shown in Figure 5A, shear stress induced the luciferase activity in cells cotransfected with wild-type ROCK, LIMK2, and cofilin, and this shear-induced activity was blocked in cells overexpressing the various negative mutants (Figure 5B).

Shear Stress Activation of SREBP2 Depends on S2P but not Caspase-3

RhoA is capable of inducing apoptosis in different cell types such as NIH3T3 fibroblast and the human erythroleukemia K562 cell line.30 SREBP2 cleavage can be induced by apoptotic stimuli through the caspase-3 site located upstream of the S2P site.11 We investigated whether the activation of SREBP2 by shear stress could be attributable to the apoptotic cleavage at the caspase-3 site. CHO-K1 cells were transfected with the wild-type HA-SREBP2 or a mutant [ie, HA-SREBP2(DS→LQ)] lacking the caspase-3 cleavage site. The application of shear stress induced the cleavage of both HA-SREBP2 and HA-SREBP2(DS→LQ) (lane 2 versus lane 1; lane 5 versus lane 4 in Figure 6A), indicating that the effect of shear stress is independent of caspase-3. In contrast, staurosporine, which is known to mediate its action via caspase-3, caused the cleavage of only HA-SREBP2 but not HA-SREBP2(DS→LQ) (lane 3 versus lane 6 in Figure 6A). We also transfected CHO-K1 with the plasmid HA-SREBP2(NP→FL), which has a mutated S2P cleavage site.11 Shear stress resulted in the S1P cleavage product (lane 8 in Figure 6A). Staurosporine treatment resulted in an additional fragment corresponding to the caspase-3 cleavage product. To additionally confirm that the shear stress–induced SREBP cleavage involves S2P but not caspase-3 in ECs, BAECs were
transfected with 4SRE-Luc together with wild-type HA-SREBP2, HA-SREBP2(DS→LQ), or HA-SREBP2(NP→FL). As expected, shear stress caused drastic induction of luciferase activity in cells cotransfected with wild-type HA-SREBP2 or HA-SREBP2(DS→LQ) but not in those with HA-SREBP2(NP→FL) (Figure 6B).

SCAP Activation Depends on F-Actin
In response to sterol depletion, SREBPs are escorted by SCAP from the ER to the Golgi and then undergo a 2-step proteolytic cleavage catalyzed by S1P and S2P. Because shear stress activation of SREBP2 depends on S2P, it is reasonable to assume that SCAP is upstream of shear stress–activated SREBP2. Mutating Asp-443 of SCAP with an Asn, i.e., SCAP(D443N), results in resistance to sterol control. The mutant constitutively activates the ER-to-Golgi transport of SREBPs for proteolytic cleavage. We used SCAP(D443N) to test whether SCAP translocation is regulated by the Rho-ROCK-LIMK-cofilin pathway. CHO-K1 cells were transiently transfected with SCAP(D443N) and HA-SREBP2, together with pcDNA3, HA-RhoN19, Myc-ROCK-C, HA-LIMK2(KD), or Flag-cofilin(S3A). As shown in Figure 7A, the expression of SCAP(D443N) enhanced HA-SREBP2 cleavage (lane 2 versus lane 1). However, this enhancement was attenuated in cells cotransfected with HA-RhoN19, Myc-ROCK-C, LIMK2(KD), or Flag-cofilin(S3A) (lanes 3 through 6 versus lane 2), suggesting that the ER-to-Golgi translocation of SCAP/SREBP2 is the step regulated by the Rho-ROCK-LIMK-cofilin pathway. Cotransfection of SCAP(D443N) was able to increase the 4SRE-Luc activity in BAECs, which was blocked by overexpression of either LIMK2(KD) or pEF-C3 encoding C3 exoenzyme (Figure 7B).

The overexpression of LIMK1 promotes the formation of F-actin because of the LIMK phosphorylation of cofilin at
Ser-3, thus inactivating cofillin.\textsuperscript{21,22} We used the actin cytoskeletal, disrupting agents latrunculin A and cytochalasin D to examine whether the SCAP(D443N)-enhanced SREBP cleavage depends on F-actin. CHO-K1 cells were transfected with SCAP(D443N) (1 \( \mu \)g) and HA-SREBP2 (0.5 \( \mu \)g) together with pCDNA3 (1 \( \mu \)g), Myc-RhoN19 (1 \( \mu \)g), Myc-ROCK-C (1 \( \mu \)g), HA-LIMK2(KD) (1 \( \mu \)g), or Flag-cofilin(S3A) (1 \( \mu \)g) (lanes 2 through 6, respectively). In control experiments (lane 1), CHO-K1 cells were transfected with pCDNA3 (2 \( \mu \)g) and HA-SREBP2 (0.5 \( \mu \)g). After 30 hours, cell lysates were collected and subjected to immunoblotting to detect the HA-SREBP2 cleavage. B, BAECs were transfected with p4SRE-luc (1 \( \mu \)g) and \( \beta \)-gal (1 \( \mu \)g) together with pCMV5 (0.5 \( \mu \)g), HA-SREBP2(wt) (0.5 \( \mu \)g), HSV-SCAP(D443N) (1 \( \mu \)g), pEF-C3 (1 \( \mu \)g), and HA-LIMK2(KD) (1 \( \mu \)g) as indicated. The luciferase and \( \beta \)-gal activity were assayed 30 hours after transfection. The fold of induction is defined as the level of normalized luciferase activity (luciferase/\( \beta \)-gal) in the various samples relative to that in the mock transfection controls set as 1. C, CHO-K1 cells were transfected with HA-SREBP2 (0.5 \( \mu \)g) together with SCAP(D443N) (1 \( \mu \)g). In control experiments (lane 1), CHO-K1 were transfected with pCDNA3 (1 \( \mu \)g) and HA-SREBP2 (0.5 \( \mu \)g). After 36 hours, cytochalasin B (1.5 \( \mu \)mol/L) (lane 3) or latrunculin A (0.5 \( \mu \)mol/L) (lane 4) was added to the culture medium for a 3-hour incubation followed by HA-SREBP2 cleavage assays. Data are representative results from 3 separate experiments.

Figure 7. SCAP(D443N)-induced HA-SREBP2 cleavage is attenuated by F-actin disassembly. A, CHO-K1 cells were transfected with SCAP(D443N) (1 \( \mu \)g) and HA-SREBP2 (0.5 \( \mu \)g) together with pCDNA3 (1 \( \mu \)g), Myc-RhoN19 (1 \( \mu \)g), Myc-ROCK-C (1 \( \mu \)g), HA-LIMK2(KD) (1 \( \mu \)g), or Flag-cofilin(S3A) (1 \( \mu \)g) (lanes 2 through 6, respectively). In control experiments (lane 1), CHO-K1 cells were transfected with pCDNA3 (2 \( \mu \)g) and HA-SREBP2 (0.5 \( \mu \)g). After 30 hours, cell lysates were collected and subjected to immunoblotting to detect the HA-SREBP2 cleavage. B, BAECs were transfected with p4SRE-luc (1 \( \mu \)g) and \( \beta \)-gal (1 \( \mu \)g) together with pCMV5 (0.5 \( \mu \)g), HA-SREBP2(wt) (0.5 \( \mu \)g), HSV-SCAP(D443N) (1 \( \mu \)g), pEF-C3 (1 \( \mu \)g), and HA-LIMK2(KD) (1 \( \mu \)g) as indicated. The luciferase and \( \beta \)-gal activity were assayed 30 hours after transfection. The fold of induction is defined as the level of normalized luciferase activity (luciferase/\( \beta \)-gal) in the various samples relative to that in the mock transfection controls set as 1. C, CHO-K1 cells were transfected with HA-SREBP2 (0.5 \( \mu \)g) together with SCAP(D443N) (1 \( \mu \)g). In control experiments (lane 1), CHO-K1 were transfected with pCDNA3 (1 \( \mu \)g) and HA-SREBP2 (0.5 \( \mu \)g). After 36 hours, cytochalasin B (1.5 \( \mu \)mol/L) (lane 3) or latrunculin A (0.5 \( \mu \)mol/L) (lane 4) was added to the culture medium for a 3-hour incubation followed by HA-SREBP2 cleavage assays. Data are representative results from 3 separate experiments.

Discussion

Functioning as key transcription factors for sterol and fatty acid synthesis, SREBPs are tightly controlled by transport-dependent proteolytic cleavage that involves SCAP, S1P, and S2P. We have previously shown that shear stress causes SREBP cleavage in the presence of a high level of sterols. SREBP cleavage is also integrin-dependent, because the engagement of \( \beta_1 \) integrin with cognate ECM ligand or anti-\( \beta_1 \) mAb is sufficient for SREBP1 cleavage.\textsuperscript{27} These previous findings led to the present study on the underlying mechanotransduction mechanism. The new information obtained from this investigation includes the following: (1) shear stress activates the Rho-ROCK-LIMK-cofilin signaling pathway; (2) this Rho pathway may render SREBPs resistant to cholesterol regulation, similar to the activation by SCAP(D443N);\textsuperscript{32} and (3) an intact F-actin cytoskeleton is necessary for the SCAP-dependent ER-to-Golgi translocation of SREBPs (summarized in Figure 8).

Kinase activity and immunostaining assays demonstrated that shear stress induces sequential signaling through the Rho-
ROCK-LIMK-cofilin pathway (Figures 1 through 3). The use of conformation-sensitive mAbs against α,β integrins revealed that shear stress activates integrins and that preventing new binding of integrins to fibronectin with blocking anti-fibronectin mAb ablated the shear activation of Rho.33 These results indicate that the interaction of integrins with the ECM proteins is an upstream mechanosensing mechanism by which shear stress activates SREBPs via Rho. Proper regulation of Rho activity is essential for the shear stress–induced cell alignment and stress fiber formation, because either inhibition of Rho by RhoN19 or activation by RhoV14, a constitutively active mutant of Rho, decreases cell alignment.28,33 These results suggest at least a permissive role of Rho in the morphological remodeling that involves actin-based cytoskeleton. It is possible that a finely modulated Rho activity is also involved in sterol and lipid homeostasis during the remodeling.

The various negative mutants in the Rho-ROCK-LIMK-cofilin pathway blocked the promoter activity driven by the sterol regulatory element (SRE) in response to shear stress (Figures 4 and 5). These results indicate that SREBP-regulated transcription is controlled by the Rho-ROCK-LIMK-cofilin pathway. Our previous results show that shear stress induces SREBP cleavage in the presence of 25-HC.27 Inasmuch as the vascular wall is constantly exposed to the flow of whole blood that contains various lipoproteins, the shear stress activation of SREBPs in ECs via Rho represents a novel mechanism by which this mechanotransduction pathway requires SCAP, S1P, and S2P. Sterol depletion releases SCAP from the ER membrane into budding vesicles,31 thus escorting the precursors of SREBPs from the ER to the Golgi for S1P and S2P proteolytic cleavage.32 The results from transient transfections with SCAP(D443N) and the various negative mutants of Rho, ROCK, LIMK, and cofilin (Figure 7A) indicate that the ER-to-Golgi transport of SCAP is most likely the critical step for the Rho pathway.

Because cofilin (S3A) inhibits both F-actin polymerization11,22 and HA-SREBP2 cleavage (Figure 4), it is reasonable to postulate that F-actin plays a permissive role in the regulation of SREBPs. The action of F-actin may be at the level of the plasma membrane-to-ER or the ER-to-Golgi translocation. The enhanced F-actin may be used for transmitting forces from integrins to intracellular organelles such as the ER to activate the signaling machinery, as suggested by the tensegrity model.36 Another interesting hypothesis to be tested is whether the mechanical cues on the plasma membrane can be transduced through the Rho-ROCK-LIMK-cofilin pathway to the ER to induce the synthesis of sterols and lipids. If F-actin enhances the ER-to-Golgi transport of SREBP, a transporting system including F-actin cytoskeleton might be used as the “highway” and SCAP-containing budding vesicle as the “vehicle” to transport SREBPs as the “cargo” from the ER to the Golgi. The activation of the Rho-ROCK-LIMK-cofilin pathway may potentiate this cargo transport. The putative transporting system may also involve motor proteins such as myosin, because butanedione monoxime, an inhibitor of myosin function, was able to abolish the SCAP(D443N)-induced SREBP cleavage (Lin and Shyy, unpublished results, 2002).

Actin dynamics also regulate the transcriptional activity of serum response factor (SRF).37 The reporter gene driven by SRF is strongly induced by the cotransfected RhoV14 or LIMK,
which could be blocked by latrunculin. A model has been proposed that the SRF-mediated transcription is regulated by an actin-interacting coactivator or a repressor.\textsuperscript{37} Using SREBPs in the present study, we provide evidence to support another mode of transcription regulation that depends on the actin-based cytoskeleton, that is, actin can mediate the SREBP cleavage through its regulation of the ER-to-Golgi transport of SCAP. By enhancing the SREBP-mediated cholesterol metabolism, this unique mechanism may contribute to EC migration\textsuperscript{18} during some physiological processes such as the development of the vascular system.

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References


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