HEAT SHOCK PROTEIN 27 (HSP27) is a member of the mammalian small HSP family. HSP27 is expressed in a variety of tissues in the presence or absence of stress (12). HSP27 is relatively abundant in all types of cells (36), and it colocalizes with actin filaments in cardiac (24), skeletal (35), and smooth muscle (16). Evidence has shown that HSP27 is important in many cell functions, such as cell survival during stress, apoptosis mediated by Fas/APO1 receptor, and microfilament organization in response to growth factor or stress, as well as smooth muscle contraction (4, 5, 21). HSP27 has been shown to exhibit chaperone activity in vitro (17) and modulate actin filament microdynamics (2, 23). HSP27 becomes phosphorylated in response to heat shock and in response to different stimuli such as cytokines, growth factors, and peptide hormones (21).}

Landry et al. (21) have mapped the phosphorylation sites in human HSP27 and showed that mitogen-activated protein (MAP) kinase-activated protein kinase-2 (MAPKAP2 kinase) phosphorylates human HSP27 protein on Ser^{15}, Ser^{78}, and Ser^{82}. Ser^{82} appears to be the major site of in vivo phosphorylation, followed by Ser^{78} and Ser^{15}, the minor sites (21, 33). HSP27 exists as both large oligomers hypothesized to have chaperone-like activity and as smaller oligomers that bind to and cap the barbed end of microfilaments and stabilize them (35). Phosphorylation is accompanied by a decrease in the size of HSP27 oligomers. Phosphorylation of HSP27 changes the actin cytoskeleton and actin-dependent events.

Because HSP27 may be of importance in smooth muscle contraction, studies of its structure-function relationship together with its interaction with other thin- and thick-filament proteins, namely actin, myosin, and tropomyosin, will greatly complement our knowledge of the contractile machinery in smooth muscle cells.

Tropomyosins are widely distributed in virtually all eukaryotic cells. They function as a crucial part of the contractile apparatus and of thin-filament assemblies of both muscle and nonmuscle cells. Tropomyosin assembles into an α-helical coiled-coil dimer, with each molecule interacting with six or seven monomers of actin (13). Tropomyosin also binds to itself and helps wrap around the actin molecule to stabilize thin-filament assembly (31).

Although the basic molecular architecture is the same, the functions of tropomyosin vary in different tissues and cell types, as well as in their location and roles within a single cell type (31). In striated muscle, whether skeletal or cardiac, tropomyosin together with the troponin complex provides the calcium switch for the turning on and off of the actin/myosin/ATPase activity associated with contraction/relaxation. In smooth muscle, the role of tropomyosin is less well understood. In smooth muscle, calcium regulation of the phosphorylation and dephosphorylation of myosin light chains is well established, but the control by...
HSP27 PHOSPHORYLATION REGULATES SMOOTH MUSCLE CONTRACTION

Isolation of smooth muscle cells from rabbit rectosigmoid.

Smooth muscle cells of rabbit rectosigmoid were isolated as described (4). Brieﬂy, the internal anal sphincter from anesthetized New Zealand White rabbits, consisting of the distalmost 3 mm of the circular muscle layer, ending at the junction of skin and mucosa, was removed by sharp dissection. A 5-cm length of the rectosigmoid orad to the junction was dissected and digested to yield isolated smooth muscle cells. The tissue was incubated for two successive 1-h periods at 31°C in 15 ml of HEPES buffer (pH 7.4). The composition of the buffer was (in mM): 115 NaCl, 5.7 KCl, 2.0 KH2PO4, 24.6 HEPES, 1.9 CaCl2, 0.6 MgCl2, and 5.6 glucose containing 0.1% (wt/vol) collagenase (150 U/mg, Worthington CLS Type II), 0.01 (wt/vol) soybean trypsin inhibitor, and 0.184 (wt/vol) DNase/RNase, and transferred onto nitrocellulose membranes or PVDF. Proteins were identified by chemiluminescence. Autoradiography was performed on blots or dried gels using a Phospho-Imager.

Immunoprecipitation and immunoblotting. Smooth muscle cells (5 × 10^6) were either untreated or treated with C2-ceramide (0.1 μM) for up to 4 min. After treatment, cells were washed with buffer A consisting of (in mM): 150 mM NaCl, 16 Na2HPO4, 4 NaH2PO4, pH 7.4 (PBS), containing 1 mM Na3VO4, and 1.0 mCi/ml [32P]orthophosphate (0.8–1.0 mCi/ml) was added, and the cells were labeled at 37°C for 4 h.

Cell stimulation and immunoprecipitation of phosphorylated HSP27. To test the hypothesis that phosphorylation of HSP27 is an important step in smooth muscle contraction, we studied the effect of the contractile agonist C2-ceramide (0.1 μM). After radiolabeling, isolated colonic smooth muscle cells were stimulated with C2-ceramide (0.1 μM) for 30 s and 4 min with or without a 20-min preincubation with the PKC inhibitors R434 (30 μM) and calphostin C (10–8 M). The reaction was stopped by quickly removing the supernatant and placing the cells on ice. The cells were rinsed three times with ice-cold PBS, then lysed in 2% SDS. The lysate was transferred to a 1.5-ml microfuge tube, boiled for 3 min, vortexed, and centrifuged for 5 min at 12,500 g. The supernatant was then transferred to a new microfuge tube and used for the subsequent steps.

Protein content was quantiﬁed using the Bio-Rad assay reagent; an equal amount of protein was used for each sample. Each sample was brought to an equal volume and then diluted 20 times with radioimmunoprecipitation assay (RIPA) buffer of the following composition: 150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate Na, 0.5 mM Tris-HCl, and 50 mM NaF.

Immunoprecipitation was then performed at 4°C, and a monoclonal antibody to HSP27 (2B4–123) was added at a 1:50 (wt/vol) dilution and allowed to mix overnight. The next day, protein G-Sepharose (2 μl/100 μl lysate) was added, and the samples were mixed for 2 h. Samples were centrifuged at 12,500 g for 5 min, and the immunocomplexed beads were transferred to 1.5 ml Microfuge tubes after removal of the supernatant. The beads were rinsed 5 times with 1 ml of RIPA buffer containing 0.1% SDS but no NaF. The immunoprecipitates were then prepared for 1-D or 2-D SDS-PAGE.

SDS-PAGE and electrophoretic transfer. For 1-D SDS-PAGE, the samples were mixed in an equal volume of 2× sample buffer consisting of 50 mM Tris, 10% (vol/vol) glycerol, 2% (wt/vol) SDS, and 0.1% (wt/vol) bromphenol blue, pH 6.8. Proteins were separated by 12.5% or 15% SDS-PAGE and transferred onto nitrocellulose membranes or PVDF. Proteins were blotted for 1 h using 1× Tris-buffered saline (TBS) or 1× TBS supplemented with 0.05% Tween 20.
protein G-Sepharose bead slurry by rocking at 4°C for 30 min. The mixture was spun at 14,000 g for 5 min at 4°C, and 1–2 μg of anti-HSP27 monoclonal antibody was added to the resultant supernatant. The mixture was incubated at 4°C for 1 h, followed by the addition of 50 μl of protein G-Sepharose bead slurry. The mixture was further incubated at 4°C for 1 h and spun at 14,000 g for 5 min, and the supernatant was aspirated off. The pellet was washed three times with buffer A, resuspended in 25 μl of 2× sample buffer, and boiled for 5 min. Immunoprecipitates were subjected to SDS-PAGE, and proteins were electrophoretically transferred to PVDF membranes. Immunoblotting was performed using a monoclonal anti-actin antibody (1:1,000), a monoclonal anti-tropomyosin antibody, or a monoclonal anti-myosin heavy-chain antibody (1:1,000) for 1 h at 24°C. Membranes were then reacted with peroxidase-conjugated goat anti-mouse IgG (1:2,500) antibody for 1 h at 24°C. Enzymes on the membrane were visualized with luminescent substrates.

**Coverslip preparation.** Glass coverslips were washed in 70% ethanol for 30 min with gentle agitation. They were allowed to dry, placed in six-well plates, and irradiated with ultraviolet light for 30 min, then coated with 0.5% poly-l-lysine and allowed to air dry. At the end of the second enzymatic digestion, the digested tissue was washed, trypsinized, and filtered as described before. Dispersed cells were transferred to a six-well tissue culture plate and allowed to settle for 24 h on the poly-l-lysine-coated coverslips in humidified 5% CO2 environment.

**Cell fixation.** Cells were fixed with 3.5% formaldehyde in PBS for 10 min. Fixative was removed, and cells were washed twice with 100-mM glycine buffer, pH 7.4, for 5 min followed by one wash with PBS. The cells were then permeabilized by adding 3 ml of the permeabilization solution with 1% Triton X-100 to each coverslip for 10 min. After permeabilization, the cells were rinsed three times with 3-ml aliquots of PBS.

**Triple immunofluorescent labeling for actin, tropomyosin, and HSP27.** Triple labeling of actin, tropomyosin, and HSP27 was performed in either resting or contracted smooth muscle cells. Cells were treated with C2-ceramide (0.1 μM), permeabilized, and fixed. Cells were incubated for 1 h with normal goat serum, followed by three 10-min washes in PBS. Cells were then reacted with Oregon green 412 phalloidin (2 units/coverslip) for 20 min and washed with PBS three times for 5 min each time. Cells were incubated for 1 h with first primary antibody, a monoclonal mouse anti-HSP27 antibody (1:50), together with second primary antibody, a polyclonal rabbit anti-tropomyosin antibody (1:100), followed by three 10-min washes in PBS. Subsequently, the cells were incubated with first secondary antibody, 1,500 dilution of AffiniPure F(ab’2) goat anti-mouse IgG, together with second secondary antibody, 1,500 dilution Cy3-conjugated AffiniPure F(ab’2) goat anti-rabbit IgG, for 1 h, followed by three 10-min washes in PBS. Finally, the cells on the coverslip were mounted on a slide with Prolong antifade mounting reagents. The following controls were also made: 1) Cy-3 only; 2) Cy-5 only; 3) Cy-3 with first primary antibody; 4) Cy-5 with first primary antibody; 5) Cy-3 with second primary antibody, and 6) Cy-5 with second primary antibody.

**Excitation parameters for the fluorescent probes were as follows:** Oregon green excitation at 492 nm, emission at 520 nm; Cy-3 excitation at 550 nm, emission at 570 nm; Cy-5 excitation at 650 nm, emission at 670 nm. Confocal images were obtained on Noran confocal imaging system. Images were analyzed using IviVision (Silicon Graphics) and Adobe Photoshop.

**Cell culture.** Rabbit rectosigmoid was removed and washed twice in PBS with penicillin/streptomycin, 70% ethanol. Mucosa and serosa were carefully removed. Circular smooth muscle layer was washed three times in PBS with penicillin/ streptomycin, then digested in HEPES buffer containing 0.1% collagenase (150 U/mg, Worthington CLS type II) at 37°C with 5% CO2 for 2 h. At the end of the enzymatic incubation period, the tissue was washed in PBS with penicillin/streptomycin and transferred into DMEM supplemented with 10% FBS. Cell suspension was filtered through 500-μm Nitex mesh. Remaining tissue was resuspended and filtered again. Cell suspension was collected in the medium and transferred to six-well plates coated with collagen IV and cultured in a humidified 5% CO2 incubator for 7–14 days before the cells reached confluence.

**Transfection of smooth muscle cells with HSP27 mutants.** Smooth muscle cells were cultured in DMEM with 10% FBS and 3% penicillin/streptomycin on collagen IV-coated dishes as previously described. Cells were passed on the day before transfection and allowed to reach 70% confluence on the day of transfection. Cells were washed with PBS twice. 3D or 3G human HSP27 (HuHSP27) mutant cDNA were transfected into the cells using QiaGen Effectene transfection kit. Briefly, the cDNA was diluted with Buffer EC and mixed with enhancer followed by incubation at room temperature for 5 min. The DNA-enhancer mixture was well mixed with Effectene transfection reagent followed by incubation at room temperature for 10 min to allow complex formation. The transfection complex was then mixed with cell culture medium and overlaid on the cells. After 2 days of transfection, the cells were selected with G-418 (3 mg/ml) for 1–2 days. Expressions of mutant HSP27 proteins were confirmed by immunoblotting the whole cell lysates with human HSP27 specific antibody (from StressGen). The protein has been shown not to cross react with rabbit and mouse antibody.

**RESULTS**

**HSP27 Phosphorylation is Stimulated by C2-ceramide (0.1 μM) and is Inhibited by the PKC Inhibitor Calphostin C**

HSP27 was immunoprecipitated from rabbit colonic smooth muscle cells after metabolic labeling with [32P]orthophosphate and stimulation with C2-ceramide (0.1 μM). Proteins were separated in 12.5% SDS-PAGE, the gel was dried, and radioactive bands resulted in inhibition of C2-ceramide-induced (0.1 μM) phosphorylation of HSP27 (76 ± 14% inhibition of phosphorylation, n = 3, P < 0.01).
Sustained Phosphorylation of HSP27 in Colonic Smooth Muscle Cells Independent of Activation of Myosin Light Chain Kinase

We next examined the effect of C2-ceramide (0.1 \( \mu M \)) on the phosphorylation of HSP27 by isoelectric focusing followed by Western blotting using a specific monoclonal antibody (2B4–123). In untreated smooth muscle cells, all four isoforms (phosphorylated, mono-, di-, and triphosphorylated) were found, indicating a basal level of phosphorylation. C2-ceramide (0.1 \( \mu M \)) induced an increase in the di- and triphosphorylated isoforms at 30 s (134.6 \( \pm \) 23.6 and 144.4 \( \pm \) 26.3% increase, respectively, \( P < 0.05 \)) and at 4 min after stimulation (163.9 \( \pm \) 13.5 and 160.6 \( \pm \) 8.7% increase, respectively, \( P < 0.01 \)) (Fig. 2). There was also a concomitant decrease in the amount of unphosphorylated isoform both at 30 s (66.4 \( \pm \) 5.2% decrease, \( n = 4, P < 0.002 \)) and at 4 min (60.3 \( \pm \) 3.7% decrease, \( n = 4, P < 0.001 \)).

The rapidity and the kinetics of the phosphorylation of HSP27 seem to correspond to the contractile response in colonic smooth muscle cells. We next tested whether C2-ceramide-induced phosphorylation of HSP27 was mediated by activation of myosin light chain kinase. Pretreatment of the cells with the myosin light chain kinase inhibitor ML7 (20 \( \mu M \) for 30 min) had no effect on the phosphorylation of HSP27 induced by C2-ceramide (0.1 \( \mu M \)). C2-ceramide (0.1 \( \mu M \)) induced an increase in the diprophosphorylated isoforms at 4 min after stimulation (120.7 \( \pm \) 4.1% increase above control, \( P < 0.07 \)) and in the triphosphorylated isoform (157.6 \( \pm \) 1.0% increase above control, \( P < 0.001 \)). There was a concomitant decrease in the amount of unphosphorylated isoform [52.7 \( \pm \) 2.4% of control, \( P < 0.006 \) (Fig. 3)]. Data thus seem to suggest that the sustained phosphorylation of HSP27 and its role in smooth muscle contraction is independent of activation of MLC kinase but could be mediated by PKC activation in colonic smooth muscle cells.

**HSP27 Coimmunoprecipitated with Actin and with Tropomyosin**

Actin, myosin, and tropomyosin are the major contractile proteins involved in generation of contraction in smooth muscle. We have previously examined the association of HSP27 with other contractile proteins (16). HSP27 coimmunoprecipitated with actin, myosin,
and tropomyosin, as revealed by immunoblotting. The observed binding affinity between those proteins suggested the possible interactions between HSP27 and contractile proteins. We have measured changes in immunoprecipitates in response to stimulation by the contractile agonist C2-ceramide (0.1 μM). Immunoprecipitations were carried out using a mouse anti-HSP27 monoclonal antibody (2B4–123). Pretreatment of the cells with the myosin light-chain kinase inhibitor ML7 (20 μM for 30 min) had no affect on the phosphorylation of HSP27 induced by C2-ceramide. C2-ceramide induced an increase in the di-phosphorylated isoforms at 4 min after stimulation (120.7 ± 4.1% increase above control, P < 0.07) and in the triphosphorylated isoform (157.6 ± 1.0% increase above control, P < 0.01) and induced a decrease in the amount of unphosphorylated isoform (62.7 ± 2.4% of control, P < 0.01). Data thus seem to suggest that the phosphorylation of HSP27 and its role in smooth muscle contraction is independent of the activation of myosin light-chain kinase. Top, representative blot of 4 independent experiments.

HSP27 Colocalizes with Tropomyosin and Actin During Smooth Muscle Contraction

We further investigated the cytoskeletal reorganization of actin, tropomyosin, and HSP27 in response to contraction induced by C2-ceramide (0.1 μM). Smooth muscle cells were isolated and allowed to attach to poly-L-lysine-coated coverslips. The cells were stimulated with C2-ceramide (0.1 μM) and immunostained with either a polyclonal anti-tropomyosin antibody or a monoclonal anti-HSP27 antibody (2B4–123) (4). The immunolocalizations were then examined under a confocal microscope. Evaluation was carried out on optical sections of reconstructed 3-D images prepared from a Z series. Immunostaining of actin, tropomyosin, and HSP27 followed by confocal microscopy revealed that in response to C2-ceramide (0.1 μM), tropomyosin and HSP27 strongly colocalized in transverse bands, similar to previously described localization of actin with HSP27 in contracted smooth muscle cells of the colon (16). We further examined the localization of actin together with tropomyosin and HSP27. In a fully contracted cell, actin and tropomyosin reorganized their distribution. The strong colocalization of actin and tropomyosin seems very similar in shape and pattern to the colocalization of tropomyosin and HSP27. When those bands were examined under high-power (×100 oil immersion) confocal microscope lens and reconstructed digitally from the serial Z sections, the bands showed a very characteristic coiled-coil pattern (Fig. 5).
examined the association of actin with myosin in cells transfected with these two HSP27 mutants. Out of $5 \times 10^6$ cells transfected through G-418 selection, the transfection rate was estimated to be $\sim 40\%$. Transfections were successfully demonstrated by detection of the mutant HuHSP27 protein via immunoblotting of the transfected cell lysates with HuHSP27-specific antibody, which has been shown not to have any cross activity between rabbit and mouse. Lysates extracted from the transfected cells were immunoprecipitated with monoclonal mouse anti-smooth muscle cell actin antibody. The immunoprecipitates were then subjected to immunoblotting using monoclonal mouse anti-myosin heavy-chain antibody. In the cells transfected with 3D HSP27 mutant, the amount of myosin binding to actin increased compared with normal nontransfected cells. C2-ceramide-induced (0.1 $\mu$M) association of actin-myosin greatly decreased in 3G HSP27 mutant transfected cells (48.5 $\pm$ 4.4%, $P < 0.005$) (Fig. 6). Data indicated that HSP27 phosphorylation might modulate the actin-myosin interaction by regulating the thin filaments of smooth muscle. Our data suggest that in response to a contractile agonist, phosphorylation of HSP27 seems to facilitate the interaction of actin with myosin, probably as a result of a stronger association of HSP27 with tropomyosin.

**DISCUSSION**

We have examined the possible role of HSP27 and its phosphorylation during cytoskeletal reorganization in smooth muscle contraction. We have examined the association of HSP27 with tropomyosin and its effects on the interaction of actin with myosin. We propose that HSP27 may affect smooth muscle thin-filament regulation through its phosphorylation and play an active role in the contractile machinery through its association with contractile proteins in smooth muscle.

We have previously reported that preincubation of smooth muscle cells from the rabbit rectosigmoid with a monoclonal antibody to HSP27 inhibits PKC-mediated contraction induced by bombesin (4). We have demonstrated that HSP27 and MAP kinase colocalize in smooth muscle cells in response to contraction induced by bombesin (37). These findings give support to a role for HSP27 in PKC-mediated contraction. Treatment of the cells with C2-ceramide (0.1 $\mu$M) resulted in an increase in phosphorylation of HSP27 detected by autoradiography at 30 s and 4 min. This increase in phosphorylation was inhibited by preincubation of the cells with calphostin C ($10^{-6}$ M). Data suggest that inhibition of PKC leads to inhibition of HSP27 phosphorylation. The following physiological paradigm arises: is HSP27 phosphorylation required for contraction and, if so, does phosphorylation of HSP27 modulate the increase in association of contractile proteins, and thus modulation of contraction?

Fig. 4. Coimmunoprecipitation of the cytoskeletal proteins actin and tropomyosin with HSP27. Smooth muscle cells ($5 \times 10^6$) were either untreated or treated with C2-ceramide (0.1 $\mu$M) for 30 s and 4 min. The protein was extracted and immunoprecipitated with monoclonal anti-HSP27 antibody as described in MATERIALS AND METHODS. SDS-PAGE and proteins were electrophoretically transferred to polyvinylidene difluoride membranes. Immunoblotting was performed using a monoclonal anti-actin antibody (1:1,000) or a monoclonal anti-tropomyosin antibody (1:1,000) for 1 h at 24°C. The membrane was then reacted with peroxidase-conjugated goat anti-mouse IgG (1:2,500) antibody for 1 h at 24°C. Enzymes on the membrane were visualized with luminous substrates. A: the amount of tropomyosin immunoprecipitated with HSP27 increases in response to ceramide stimulus. Immunoprecipitates of HSP27 (IP) from 200 $\mu$g of protein lysate of cells either untreated or stimulated with C2-ceramide were subjected to SDS-PAGE and Western blotted with anti-tropomyosin monoclonal antibody (IB). C2-ceramide induced a significant and sustained (30 s and 4 min) increase in the association of HSP27 with tropomyosin. Inset, representative blot of 4 independent experiments. B: the amount of actin immunoprecipitated with HSP27 increases in response to ceramide stimulation. Immunoprecipitates of HSP27 (IP) from 200 $\mu$g of protein lysate of untreated cells or cells stimulated with C2-ceramide were subjected to SDS-PAGE and Western blotted with anti-actin monoclonal antibody (IB). C2-ceramide induced a significant and sustained (30 s and 4 min) increase in the association of HSP27 with tropomyosin. Inset, representative blot of 4 independent experiments.
During prolonged contraction of some smooth muscle types, the association between intracellular calcium concentration, LC20 phosphorylation levels, and cross-bridge cycling grades and force has been reported (7, 25). Others have shown that in a calcium-free medium, PKC-dependent translocation of cytosolic MAP kinase to the membrane occurs before contraction, followed by a second redistribution of MAP kinase to the contractile filaments during cell contraction (20). Sustained smooth muscle contraction is associated with activation of MAP kinase. MAP kinase has been implicated in the maintenance of force in gastrointestinal and vascular smooth muscle cells (1, 8, 18, 37). MAP kinase has been shown to activate a model kinase MAPKAP2.
HSP27 phosphorylation regulates smooth muscle contraction

The evidence suggests that HSP27 may serve as a link between signal transduction pathways leading to contraction and the final contractile apparatus. We have shown that HSP27 can immunoprecipitate with tropomyosin (16). We have also shown that HSP27 can immunoprecipitate with other cytoskeletal proteins including actin, myosin, and caldesmon (15). However, such bindings could be indirect. Data indicate that during smooth muscle contraction, there was increased association between HSP27 and tropomyosin, suggesting that HSP27 might interact with tropomyosin to regulate smooth muscle contraction. Our data, using immunofluorescent dual labeling of tropomyosin and HSP27 followed by confocal microscopy, indicated that in the control relaxed state, tropomyosin and HSP27 are distributed along the longitudinal axis of the smooth muscle cell in a fiber-like manner. C2-ceramide (0.1 μM) induced sustained smooth muscle contraction and the association of tropomyosin with HSP27 (19).

In this paper, we propose a model in which HSP27 binds to tropomyosin and colocalizes extremely well with the coiled-coil structure of tropomyosin, forming a coiled-coil structure in smooth muscle cells that were shortened and contracted by the contractile agonist C2-ceramide.

HSP27 has been shown to regulate the microfilaments in cells. Miron et al. (26) have shown that HSP27 could affect the in vitro polymerization kinetics of actin. It was proposed that HSP27 binds to actin filament barbed ends and caps the filament end to stabilize the filament. Overexpression of HSP27 in a variety of cultured cell lines results in increased polymerized actin and changes in functions believed to actively involve microfilaments (10, 14). Phosphorylation has been shown to modulate the activity of HSP27 by many studies. HSP27 phosphorylation has been suggested to relate the interaction of HSP27 with microfilaments (2). We have shown that there was increased association between tropomyosin and phosphorylated HSP27, suggesting that HSP27 may regulate tropomyosin through its phosphorylation.

Is the phosphorylation of HSP27 necessary for complexing with other proteins? We have investigated how HSP27 phosphorylation affected actin-myosin interaction. Data suggest that by overexpressing HSP27 phosphorylation mutants in smooth muscle cells, HSP27 phosphorylation is important in the association of actin...
with myosin as immunoprecipitation and immunoblotting data revealed (Fig. 6).

We have presented data that indicate a role for HSP27 and its phosphorylation in smooth muscle thin-filament regulation during smooth muscle contraction. We propose a model whereby phosphorylated HSP27 may undergo conformational changes that lead to the binding of HSP27 with both actin and tropomyosin. When HSP27 is unphosphorylated, the tropomyosins bind to the actin molecules and occupy the potential myosin binding sites on actin. On phosphorylation, HSP27 may bind to tropomyosin and pull tropomyosin away from the myosin head-binding site into the F-actin groove. As a result, the actin molecules are more available to myosin heads. Thus HSP27 may function as a facilitator in thin-filament regulation of smooth muscle contraction.

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