Changes in actin dynamics influence diverse cellular processes and couple the actin-based cytoskeleton to changes in gene transcription. Members of the Rho GTPase family regulate cytoskeletal organization by stimulating actin polymerization and stress fiber formation when activated by extracellular signaling. The transcripational activity of serum response factor (SRF) is stimulated in response to changes in actin dynamics and Rho signaling, but the proteins that mediate this phenomenon have not been fully identified. We describe a novel, evolutionarily conserved actin-binding protein, called STARS (striated muscle activator of Rho signaling), that is expressed specifically in cardiac and skeletal muscle cells. STARS binds to the I-band of the sarcomere and to actin filaments in transfected cells, where it activates Rho-signaling events. STARS stimulates the transcripational activity of SRF through a mechanism that requires actin binding and involves Rho GTPase activation. STARS provides a potential mechanism for specifically enhancing Rho-dependent transcription in muscle cells and for linking changes in actin dynamics to gene transcription.

The actin cytoskeleton influences diverse cellular processes, including motility, mitosis, contractility, cytokinesis, endocytosis, and secretion (1–3). Actin also participates in numerous transmembrane signaling systems by forming complexes with cell adhesion molecules and receptors (4, 5). In addition, actin has been implicated in the control of gene transcription through its direct association with chromatin-remodeling complexes (6) and through indirect mechanisms mediated by changes in cytoskeletal actin dynamics (7).

Actin exists in monomeric (G-actin) and polymerized (F-actin) forms. The distribution of actin between these two forms is tightly regulated and is influenced by numerous actin-binding proteins that control actin dynamics by severing (i.e. actin-depolymerizing factor/cofilin), cross-linking (i.e. α-actinin, tropomyosin), and capping (i.e. tropomodulin at the point ends and capZ at the Z-line) actin (8). Members of the Rho GTPase family regulate cytoskeletal organization by stimulating actin polymerization and stress fiber formation when activated by extracellular signaling (9). A number of Rho effector molecules, including Rho kinase, mDia, and phosphatidylinositol phosphate 5-kinase, also participate in cytoskeletal organization (10–14).

Recent studies showed that RhoA signaling stimulates the transcriptional activity of serum response factor (SRF) through a mechanism mediated by changes in actin dynamics (7, 15). SRF is a MADS-box transcription factor that regulates serum-inducible and muscle-specific gene expression by binding to a consensus sequence known as a CARG box, CC(ATT)GG (16, 17). Members of the myocyte enhancer factor-2 family of MADS-box transcription factors, which regulate muscle cell differentiation (18), are also stimulated by RhoA signaling (19). Consistent with a role in the activation of SRF and myocyte enhancer factor-2 transcription factors, Rho signaling has been shown to be required for muscle cell differentiation (20). Rho signaling has also been shown to activate members of the GATA family of transcription factors during cardiomyocyte hypertrophy (21). In light of the requirement of Rho signaling during muscle cell differentiation and hypertrophy, it is tempting to speculate as to the existence of muscle-specific regulators of Rho signaling.

Here, we describe a novel actin-binding protein, striated muscle activator of Rho signaling (STARS), identified in a differential cDNA screen for unknown genes expressed in the early embryonic heart. STARS is associated with the I-band of the sarcomere and with actin stress fibers in transfected cells. STARS contains a unique, evolutionarily conserved domain that stimulates SRF-dependent transcription through a mechanism that requires actin polymerization and Rho GTPase activation. These findings suggest that STARS acts as a muscle-specific transducer of cytoskeletal signals that stimulate Rho signaling, thereby activating SRF-dependent transcription.

**MATERIALS AND METHODS**

**Differential cDNA Cloning** — For subtractive screening by representational difference analysis, hearts and the region of the embryodorsal to the heart, including the neural fold and the first three somites, were dissected from embryonic day (E)-8.25 mouse embryos. Total RNA was isolated from the heart and the dorsal embryonic region using TRIZOL (Invitrogen). mRNA was purified using an mRNA purification kit (Amersham Biosciences) and converted into double-strand cDNA by the Superscript choice system for cDNA synthesis (Invitrogen). For subtraction screening between heart (Tester) and other embryonic parts (Driver), we employed the representational difference analysis method described above. The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF504061 and AF503817.

1 The abbreviations used are: SRF, serum response factor; MADS, Mcm1, Agamous, Deficiens, and SRF; DP, differential product; PBS, phosphate-buffered saline; GST, glutathione S-transferase; E, embryonic day; BSA, bovine serum albumin; TRITC, tetramethylrhodamine isothiocyanate; CD, cytochalasin D; LB, latrunculin B; GEF, guanine nucleotide exchange factor; RACE, rapid amplification of cDNA ends.
scribed by Hubank and Schatz (22). Briefly, the cDNA from both tissues was digested with DpnII and ligated with an R-linker (the annealed oligonucleotides were 5'-CATGCTGGGCTGA-3' and R-Bgl-24 (5'-AGGACCTCTCCAGGCCTCAGGGCA-3')). Using R-Bgl-24 as primer, cDNA was amplified by PCR and digested with DpnII again. cDNA and DpnII digested cDNA was ligated with a J-linker (the annealed oligonucleotides were J-Bgl-12 (5'-GATCTGTT-CATG-3') and J-Bgl-24 (5'-ACCGACGTCTACCTTACGAAACA-3')). Tester ligated with J-linker and Driver cDNAs (ratio 1:100) were hybridized at 67 °C for 20 h. PCR reactions were then performed by primer J-Bgl-24 to yield a PCR product referred to as DPI (differential product). DPI was digested with DpnII and new tester ligated with a N-linker (the annealed oligonucleotides were N-Bgl-12 (5'-GATCTTTC-CCATCG-3') and N-Bgl-24 (5'-AGGACAACTGTGGTACCGAAGGA-3')). N-Linker-ligated DPI was hybridized with Driver (ratio 1:800) and amplified by PCR two times using N-oligonucleotides as primer. Linkers for tester were replaced with J-oligonucleotides in the third round and N-oligonucleotides in the fourth round of hybridization. The final PCR products, referred to as DPIV, were cloned into the TA cloning vector (pGEMT-easy, Promega), and inserted DPIV fragments were amplified by PCR using N-Bgl-24 as primers to make two identical dot blots. One dot blot was hybridized with tester probe, and the other was hybridized with driver probe to confirm the differential expression of DPIV fragments.

To obtain a full-length STARS cDNA, an E10.5 mouse heart cDNA library (Stratagene) was screened using a 342-bp cDNA fragment isolated by representational difference analysis. The longest positive clone contained a 375-amino acid open reading frame without a stop codon 5' of the first methionine in the sequence. To further obtain 5' sequences, adult mouse heart and skeletal muscle cDNA libraries (CLONTECH) were screened with a 400-bp cDNA fragment from the 5' end of the longest cDNA as a probe. 5' Rapid amplification of cDNA ends (5'-RACE) was also performed using the SMART 5'-RACE kit (CLONTECH) and the RLM first choice 5'-RACE kit (Ambion) following the manufacturer's instructions. 5' rapid amplification of cDNA ends was performed with mRNA from human skeletal muscle (CLONTECH) and from mouse heart and skeletal muscle. There were no stop codons upstream of the first methionine.

**Northern Blot Analysis—**Northern blot analysis was performed using mouse and human multiple tissue Northern blots (CLONTECH). Labelled probes were prepared from a full-length mouse STARS cDNA and a partial human cDNA. Hybridizations were performed in Quick-Hyb (CLONTECH). In situ Hybridization—*RNA probes corresponding to the sense and the antisense strands of the STARS cDNA were prepared. In situ hybridization was performed on sagittal sections of mouse embryos as described (23).**

**STARS Expression Plasmids—**STARS expression constructs were generated using the pcDNA3.1 mammalian expression vector (Invitrogen), which was modified to contain an amino-terminal Myc tag. Constitutively active RhoA (L63) and C3 transferase in Ppk5 were generously provided by Alan Hall (University College, London, UK). The SM22-luciferase reporter contained the 1343-bp promoter (24). The smooth muscle α-actin-luciferase reporter contained 1 kb of promoter region, the first untranslated exon, and the first intron ligated into the pBLC3 basic vector (Promega). The 4cos reporter contained four tandem copies of the f-cos CarG box with flanking sequences as described (25). CMV-lacZ (Promega) was used as an internal control for transfection efficiency.

**Transient Transfections, Reporter Assays, and Immunofluorescence Microscopy—**Neonatal rat cardiomyocytes were isolated as described previously (26). COS cells were maintained in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. Eighteen hours after plating, medium was changed to serum-free medium, and transfections were performed at 50% confluence using FuGENE 6 reagent (Roche Molecular Biochemicals) following the manufacturer's instructions. Each transfection used 0.15 µg of plasmid DNA for the reporter assays and 0.5–1 µg for immunohistochemistry. Cytochalasin D 2 µg/ml (Sigma-Aldrich), and Y-27632 75 µM (Toxic) were added directly just after transfection. Latrunculin B (0.5 µM; dissolved in 10% ethanol, Calbiochem) was added 12 h after transfection, and 10% ethanol (final concentration 0.01%) was added to control cells. Cells were harvested 36 h after transfection, and luciferase and β-galactosidase activity were measured. All transfection assays were performed at least three times with duplicates for each assay.

For immunofluorescence, cells were rinsed with PBS, incubated in 0.1% Triton X-100 in PBS for 5 min on ice, and fixed in 4% paraformaldehyde for 10 min on ice. For rat neonatal cardiomyocytes, 1% Triton X-100 in PBS was used. Cells were blocked for 30 min at room temperature in 2.5% bovine serum albumin (BSA) in PBS for COS cells and in 1% BSA plus 3% goat serum in PBS for rat cardiomyocytes. Then, cells were incubated with first antibody diluted in 0.5% BSA in PBS for 30–60 min at room temperature or overnight at 4 °C. Anti c-Myc monoclonal antibody 9E10 (Santa Cruz) was used at a 1:2000 dilution. Other antibodies were anti-STARS antibody (1:50), α-actin monoclonal antibody (1:400; Sigma-Aldrich), α-tubulin antibody (1:2000; Sigma-Aldrich), and vimentin antibody (1:500; Sigma-Aldrich). Secondary antibodies were anti-mouse/rabbit IgG fluorescein isothiocyanate or Texas Red (Vector) used at a 1:200 dilution incubated with or without phalloidin-TRITC (1:500; Molecular Probes).

Western Blot and Co-immunoprecipitation Assay—Immunoprecipitation and Western blot analysis was performed as described previously (27). For immunoprecipitation, cells were incubated with 1 µg of anti-c-Myc polyclonal antibody A14 (Santa Cruz). Western blots were performed with anti-actin monoclonal antibody C4 (1:100; Chemicon) or anti-ε-c-Myc monoclonal antibody 9E10 (1:1000; Santa Cruz) followed by incubation with secondary antibody horseradish peroxidase-conjugated anti mouse-IgG (Santa Cruz). To detect signals, blots were incubated with Transluminol (Santa Cruz) for 1 min and exposed to film (Eastman Kodak Co.).

**GST-STARS Purification and Sedimentation Assays—**A cDNA encoding the full-length STARS open reading frame was cloned in-frame into pGEMEX (28). Blasticidin containing plasmids (pGEMEX-1) were transfected into COS7 cells using the pcDNA3.1 mammalian expression vector fused to GST. For immunostaining and Western blots, IgG was purified from antisera with protein A-Sepharose beads (Zymed Laboratories, Inc.).

**RESULTS**

**Evolutionary Conservation of STARS Proteins—**We performed a differential cDNA screen for novel genes expressed in the mouse heart tube at E8.25 but not in other regions of the E8.25 embryo (see “Materials and Methods”). Positive clones representing potential heart tube–specific genes were used for whole-mount in situ hybridization to confirm their cardiac specificity. One of the cardiac-specific cDNAs isolated in the screen encoded a novel 375-amino acid protein that we refer to as striated muscle activator of Rho signaling (STARS), because it is expressed specifically in striated muscle where it binds and activates Rho GTPase (see below). The predicted protein motif was also identified in the zebrafish Expressed Sequence Tag data base (accession number AI721847) (Fig. 1). No other related genes were identified in the searches of the mouse and human genome sequences. Genomic sequences with the potential to encode proteins with high homology to the carboxyl-terminal 142 amino acids of STARS were also identified in Caenorhabditis elegans and Drosophila melanogaster (accession numbers F36F2.1 and T04A8.4, respectively) (Fig. 1).

**STARS Is Expressed Specifically in Cardiac and Skeletal Muscle—**Consistent with the cDNA subtraction scheme, STARS transcripts were detected by in situ hybridization specifically in the striated heart tube at E8.75 (Fig. 2A). Thereafter, STARS expression was maintained in the heart and was also detected in skeletal muscle after E10.5 (data not shown). Northern analysis of adult tissues revealed three STARS transcripts in mouse heart and skeletal muscle and two transcripts in these human tissues (Fig. 2B). Sequencing of multiple independent cDNAs revealed only a single open reading frame with
no evidence for alternative splicing within the protein-coding region. Therefore, we believe the multiple transcripts reflect the presence of different 3'-untranslated sequences.

**STARS Localization in Cardiomyocytes**—Western blot analysis of adult mouse heart extracts with an anti-STARS antibody revealed a single species of 45 kDa, similar to the predicted size of STARS (Fig. 3A). This band comigrated with a Myc-tagged STARS protein expressed in transfected COS cells.

To determine the subcellular distribution of STARS, rat primary cardiomyocytes were immunostained with the STARS antibody (Fig. 3B, a and d). STARS staining showed a periodicity reminiscent of sarcomeric localization. The sarcomeric localization of STARS abuts the Z-line on both sides, as demonstrated by a partial overlap with α-actinin (Fig. 3B, b and c, e and f). This periodicity demonstrates that STARS is localized to the I-band of the sarcomere. In addition to I-band localization, a portion of STARS localizes to sarcomeric structures between Z-lines (Fig. 3B, c and f). A schematic of STARS localization is depicted in Fig. 3C.

**Mapping the Actin Binding Domain of STARS**—We also examined the subcellular localization of a Myc-tagged STARS protein in transiently transfected COS cells. STARS colocalized with actin, which is marked by phalloidin staining (Fig. 4A, a–f). Interestingly, in a small percentage of cells, STARS was localized to the nucleus (data not shown).

To determine whether a GST-STARS fusion protein was able to bind actin, we performed in vitro actin sedimentation assays. In the absence of actin, GST-STARS was predominantly contained in the supernatant after centrifugation (Fig. 4B, lanes 1 and 2). However, in the presence of actin, GST-STARS was present almost exclusively in the actin pellet (lanes 3 and 4). As a positive control, purified actinin was used and, as expected, was contained almost exclusively in the actin pellet (lanes 5 and 6). In contrast, BSA failed to co-sediment with actin under these conditions. These results demonstrate that STARS directly associates with actin and appears to enhance actin polymerization and/or stabilize polymerized actin (compare lanes 3 and 4 to lanes 7 and 8).

**STARS Stimulates SRF-dependent Transcription**—Recent studies show that stabilization of the actin cytoskeleton stimulates the transcriptional activity of SRF (7, 15). To examine whether STARS might participate in a signaling pathway between the cytoskeleton and the nucleus, we tested whether the promoter of the SM22 gene, which is regulated by SRF in muscle cells (29, 30), was responsive to STARS. Remarkably, STARS stimulated the expression of an SM22-luciferase re-
porter by 40-fold in transiently transfected COS cells (Fig. 6A). Mutation of the CArG boxes in the SM22 promoter abolished responsiveness to STARS, demonstrating the involvement of SRF in this response. Stimulation of SRF activity is not a general property of actin-binding proteins, as H9251-actinin has no effect on SRF activity (data not shown). STARS also weakly stimulated the H9251-smooth muscle actin promoter, which is regulated by SRF (31), and it activated an E1b promoter linked to four tandem copies of the CArG box and flanking sequences from the c-fos promoter (Fig. 6B).

Comparisons of the complete set of STARS deletion mutants showed a direct correlation between the ability to bind actin and to stimulate SM22 transcription (Figs. 5 and 6C). The conserved carboxyl-terminal region of STARS was necessary and sufficient to stimulate SRF activity, as demonstrated by the finding that mutant C142 was as effective as the full-length protein in activating SM22-luciferase.

Effects of Actin-depolymerizing Agents on SM22 Promoter Activation by STARS—To examine whether STARS stimulates SRF activity via its effects on actin dynamics, we treated COS cells with cytochalasin D (CD), which prevents actin polymerization (32), and latrunculin B (LB), which sequesters monomeric actin (33). STARS-transfected cells, only short frag-
STARS on the effects of a constitutively active RhoA mutant (L63) and STARS—G-actin monomers (33). Whereas CD dimerizes G-actin (34), LB sequesters are likely to reflect their differential effects on the pool of by the actin cytoskeleton. The opposing effects of CD and LB affected with STARS (Fig. 6D). These findings suggested that STARS-mediated activation by 55% (40-fold) versus that in cells transfected with the indicated reporters without STARS. Error bars indicate S.E.

FIG. 6. STARS stimulates SRF activity through a Rho-dependent mechanism. A, COS were transiently transfected with an SM22-luciferase reporter or the same reporter containing mutations in the CArG boxes within the SM22 promoter (mutSM22) and a STARS expression vector. B, COS cells were transiently transfected with the indicated reporter plasmids with and without a STARS expression plasmid. C, COS cells were transiently transfected with SM22-luciferase and expression plasmids encoding the indicated STARS mutants. D, COS cells were transiently transfected with SM22-luciferase in the presence and absence of STARS and the indicated agents. E, COS cells were transiently transfected with SM22-luciferase and a STARS or RhoA(L63) expression vector. F, COS cells were transiently transfected with SM22-luciferase and a STARS expression vector in the presence of Y-27632 or a C3 expression vector. Transfections were performed as described under “Materials and Methods.” Luciferase activity was determined on cell extracts and is expressed as the level of activity relative to that in cells transfected with the indicated reporters without STARS.

ments of disrupted stress fibers were observed in the presence of 2 μM CD or 0.5 μM LB (data not shown). STARS activated SM22-luciferase ~40-fold (Fig. 6D). In the absence of STARS, CD itself increased SM22 promoter activity by 8-fold, consistent with previous results (7, 15). However, CD inhibited STARS-mediated activation by 55% (40-fold versus 18-fold). LB treatment abolished SM22-luciferase activity in cells transfected with STARS (Fig. 6D). These findings suggested that transcriptional activation by STARS is mediated in large part by the actin cytoskeleton. The opposing effects of CD and LB are likely to reflect their differential effects on the pool of G-actin; whereas CD dimerizes G-actin (34), LB sequesters G-actin monomers (33).

Involvement of RhoA in Transcriptional Activation by STARS—In light of the ability of RhoA to stimulate SRF activity by promoting actin polymerization (7, 15), we compared the effects of a constitutively active RhoA mutant (L63) and STARS on SM22 promoter activity. As shown in Fig. 6E, STARS and RhoA L63 activated the SM22 promoter to comparable levels and together stimulated activity to higher levels.

Rho signaling is inhibited by the Rho kinase inhibitor Y-27632, which prevents stress fiber formation (11), and C3 transferase, which specifically ADP-ribosylates RhoA (35). Treatment of COS cells with Y-27632 (75 μM) or transfection with a C3 transferase expression plasmid reduced the stimulatory activity of STARS on the SM22 promoter by 55 and 88%, respectively (Fig. 6F). Together, these results suggest that STARS activation of transcription is mediated at least in part by a Rho-dependent mechanism.

DISCUSSION

STARS is a novel and evolutionarily conserved actin-binding protein expressed specifically in striated muscle. STARS localizes to the sarcomere of cardiomyocytes and to actin stress fibers in transfected cells. Stabilization of the actin cytoskeleton by STARS stimulates SRF-dependent transcription through a mechanism that involves RhoA signaling.

The Actin Binding Functions of STARS—STARS contains no recognizable protein motifs and represents a new type of sarcomeric actin-binding protein. Deletion mutants of STARS indicate that there are two separate regions that work in concert to bind actin. The high conservation of the actin binding regions between vertebrate and invertebrate SRF proteins suggests that this activity has been evolutionarily conserved. It should also be noted that STARS associates with the actin cytoskeleton in transfected cells but is restricted predominantly to the I-band in striated muscle. This raises the possibility that muscle-specific proteins serve to localize STARS to the I-band. STARS also partially overlaps the Z-line, suggesting the potential involvement of one or more Z-line proteins in restricting its distribution.

STARS expression is initiated in cardiac and skeletal muscle during the period of myofibrillogenesis when the myofibrillar components become assembled into the functional sarcomere (36, 37). Considering the timing of its expression and its actin binding properties, it is tempting to speculate that STARS may participate in sarcomere assembly by inducing actin polymerization and cross-linking during striated muscle development.

Increased expression of STARS during myogenesis may enhance Rho signaling, which has been shown to be required for muscle differentiation (38).

Stimulation of SRF-dependent Transcription by STARS—Consistent with recent studies implicating actin dynamics in the control of SRF-dependent transcription (7, 15, 39), STARS stimulates the activity of SRF. The conserved carboxyl-terminal region of STARS is both necessary and sufficient for SRF activation and actin binding; the correlation between these activities argues that the effects of STARS on SRF are coupled to its effects on the cytoskeleton. Stimulation of SRF activity is observed with native muscle promoters as well as with an artificial promoter containing multimers of CArG boxes. Thus, we conclude that the transcription-enhancing properties of STARS are directed specifically at SRF rather than another transcription factor that cooperates with SRF.

What is the mechanism for activation of SRF by STARS? A working model of a potential mechanism of action of STARS within the context of the regulation of actin dynamics is shown in Fig. 7. Actin treadmilling determines the relative distribution of the monomeric G-actin and polymerized F-actin states. Previous studies suggested that G-actin suppresses the activity of SRF (7). Our results suggest that STARS activates SRF by relieving this repressive influence (as a result of its ability to stimulate actin polymerization) and thereby reduce the G-actin pool. Such a mechanism would account for the ability of latrun-
cates Rho kinase as a downstream effector of STARS. However, the finding that Y-27632 only partially inhibited STARS activity also suggests the involvement of other Rho effectors in the mechanism for STARS action.

Rho GTPases act as molecular switches that cycle between inactive (GDP-bound) and active (GTP-bound) conformations. Guanine nucleotide exchange factors (GEFs) activate Rho GTPases by accelerating GDP/GTP exchange, whereas GTPase-activating proteins stimulate GTP hydrolysis (14). Although STARS acts at least in part via a RhoA-dependent signaling pathway, STARS does not show sequence homology with GEFs nor does it contain a nucleotide binding domain, suggesting that it does not act directly on Rho A. Intriguingly, however, a novel GEF, called p63RhoGEF, was recently shown to associate with the I-band of the sarcomere and to stimulate RhoA activity (46). The colocalization of p63RhoGEF and STARS and the involvement of both proteins in Rho activation raises the interesting possibility that these proteins may interact or that STARS may influence p63RhoGEF activity.

Rho signaling plays an important role in muscle gene expression and myogenesis. Previous studies have shown that RhoA activates SRF-dependent transcription, thereby up-regulating MyoD and triggering the differentiation cascade (47). Activation of RhoA by Gq-coupled receptor agonists also induces premyofibrils, myofibril organization, and atrial natriuretic factor expression in primary cardiomyocytes (48–50). Of course, stimulation of SRF activity by RhoA does not require STARS, since Rho can activate SRF in nonmuscle cells in which STARS is not expressed. Although it is formally possible that RhoA acts in parallel to STARS and that the action of RhoA on the cytoskeleton promotes STARS-induced gene expression rather than acting as a downstream effector for STARS, we feel this is less likely because STARS can activate SRF in serum-starved fibroblasts in which RhoA does not induce actin bundling (51).

**Linking the Cytoskeleton and the Sarcomere to Muscle Gene Expression**—STARS expression is maintained in adult cardiac and skeletal muscle and is dramatically up-regulated during hypertrophic growth of the heart in response to calcineurin activation as well as pressure-overload, which is associated with calcineurin activation (52). Sarcomere organization is a hallmark of cardiac hypertrophy. During hypertrophic growth of cardiomyocytes, STARS might organize new myofibrils, whereas extreme overexpression might result in disorganization of actin bundles with resulting cardiac dysfunction, as observed in failing hearts.

The integrity of the cytoskeleton and sarcomere has a profound influence on gene expression and growth of muscle cells. The ability of STARS to activate Rho signaling through actin association may provide a potential link between myocyte structure and the program for muscle gene expression. In light of the critical role of Rho signaling in mechanical stress-induced hypertrophy (53) and the function of the \( Z \)-line in mechanosignaling (54), the potential involvement of STARS in mechanosignaling during development and disease of striated muscle cells is an especially interesting issue for the future.

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**REFERENCES**

1. Berridge, K., and Chrzanoska-Wodnicka, M. (1996) *Annu. Rev. Cell Dev. Biol. 12*, 463–518
2. Schmidt, A., and Hall, M. N. (1998) *Annu. Rev. Cell Dev. Biol. 14*, 305–338
3. Pantaloni, D., Le Clainche, C., and Carlier, M. F. (2001) *Science 292*, 1502–1506

\( ^{2} \) A. Arai, J. Spencer, and E. N. Olson, unpublished results.
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4. Juliano, R. L., and Haskill, S. (1993) J. Cell Biol. 120, 577–585
5. Calderwood, D. A., Shattil, S. J., and Ginsberg, M. H. (2000) J. Biol. Chem. 275, 22807–22810
6. Rando, O. J., Zhao, K., and Crabtree, G. R. (2000) Trends Cell Biol. 10, 92–97
7. Sotirioupolos, A., Ginis, D., Copeland, J., and Treisman, R. (1999) Cell 98, 159–169
8. Cooper, J. A., and Schafer, D. A. (2000) Curr. Opin. Cell Biol. 12, 97–103
9. Ridley, A. J., and Hall, A. (1992) Cell 70, 389–399
10. Kaibuchi, K., Kuroda, S., and Amano, M. (1999) Annu. Rev. Biochem. 68, 459–486
11. Maekawa, M., Ishizaki, T., Boku, S., Watanabe, N., Fujita, A., Iwamatsu, A., Obinata, T., Ohashi, K., Mizuno, K., and Narumiya, S. (1999) Science 285, 895–898
12. Narumiya, S., Ishizaki, T., and Watanabe, N. (1997) FEBS Lett. 410, 68–72
13. Yamamoto, M., Hilgemann, D. H., Feng, S., Bito, H., Ishihara, H., Shibasaki, Y., and Yin, H. L. (2001) J. Cell Biol. 152, 867–876
14. Van Aelst, L., and D'Souza-Schorey, C. (1997) Genes Dev. 11, 2295–2322
15. Mack, C. P., Somlyo, A. V., Hautmann, M., Somlyo, A. P., and Owens, G. K. (2001) J. Biol. Chem. 276, 341–347
16. Treisman, R. (1995) Nature 376, 468–469
17. Treisman, R. (1995) EMBO J. 14, 4905–4913
18. Black, B. L., and Olson, E. N. (1998) Annu. Rev. Cell Dev. Biol. 14, 167–196
19. Marnissi, M. J., Chiarriello, M., and Gutkind, J. S. (2001) Genes Dev. 15, 535–553
20. Takano, H., Komuro, I., Oka, T., Shiigiima, I., Hiroi, Y., Mizuno, T., and Yazaki, Y. (1998) Mol. Cell. Biol. 18, 1586–1589
21. Yanazume, T., Hasegawa, K., Wada, H., Morimoti, T., Abe, M., Kawamura, T., and Sasayama, S. (2001) J. Biol. Chem. 277, 8618–8625
22. Hubank, M., and Schutz, D. G. (1999) Methods Enzymol. 303, 325–349
23. Benjamin, L. J., Shelton, J., Garry, D. J., and Richardson, J. A. (1997) Dev. Dyn. 208, 75–84
24. Li, L., Miano, J. M., Mercer, B., and Olson, E. N. (1996) J. Cell Biol. 132, 1–11
25. Chang, P. S., Li, L., McNally, J., and Olson, E. N. (2001) J. Biol. Chem. 276, 17209–17212
26. Nicol, R. L., Froy, N., Pearson, G., Cobb, M., Richardson, J., and Olson, E. N. (2001) EMBO J. 20, 2757–2767
27. Lu, J., McKinsey T. A., Zhang C. L., and Olson E. N. (2000) Mol. Cell 6, 233–244
28. Guan, K. L., and Dixon, J. E. (1991) Annu. Rev. Biochem. 59, 289–311
29. Li, L., Liu, Z., Mercer, B., Overbeek, P., and Olson, E. N. (1997) Dev. Biol. 187, 311–321
30. Kim, S., Ip, H. S., Lu, M. M., Clendenin, C., and Parmacek, M. S. (1997) Mol. Cell. Biol. 17, 2266–2278
31. Mack, C. P., and Owens, G. K. (1999) Circ. Res. 84, 852–861
32. Sampath, P., and Pollard, T. (1991) Biochemistry (Moscow) 30, 1973–1980
33. Morton, W. M., Ayas, K. R., and McLaughlin, P. J. (2000) Nat. Cell Biol. 2, 376–378
34. Goddette, D. W., and Frieden, C. (1986) J. Biol. Chem. 261, 15970–15973
35. Nemoto, Y., Nanai, T., Teru-uchi, T., Ushikubi, F., Morii, N., and Narumiya, S. (1992) J. Biol. Chem. 267, 20916–20920
36. Ehler, R., Ino, T., Hammerle, S. P., Komiyama, M., and Perriard, J. C. (1999) J. Cell Sci. 112, 1529–1539
37. Gregorio, C. C., and Antin, P. B. (2000) Trends Cell Biol. 10, 355–362
38. Wei, L., Zhou, W., Croissant, J. D., Johansen, F. E., Prywes, R., Balasubramanyam, A., and Schwartz, R. J. (1998) J. Biol. Chem. 273, 30287–30294
39. Wei, L., Wang, R., Carson, J. A., Agan, J. E., Imanaka-Yoshida, K., and Schwartz, R. J. (2001) FASEB J. 15, 785–796
40. Wada, A., Fukuda, M., Mishima, M., and Nishida, K. (1999) EMBO J. 17, 1635–1641
41. Zhao, K., Wang, W., Rando, O. J., Xue, Y., Swiderek, K., Kuo, A., and Crabtree, G. B. (1999) Cell 95, 625–636
42. Chen, C. Y., and Schwartz, R. J. (1996) Mol. Cell Biol. 16, 6372–6384
43. Belaguli, N. S., Sepulveda, J. L., Nigam, V., Charon, F., Nemer, M., and Schwartz, R. J. (2000) Mol. Cell. Biol. 20, 7550–7558
44. Morin, S., Paradis, P., Aries, A., and Nemer, M. (2001) Mol. Cell. Biol. 21, 1036–1044
45. Wang, D., Chang, P. S., Wang, Z., Sutherland, L., Richardson, J. A., Small, E., Krieg, P. A., and Olson, E. N. (2001) Cell 105, 851–862
46. Souchet, M., Portales-Casas, E., Mazauric, D., Schmidt, S., Leger, I., Jave, J. L., Robert, P., Berrebi-Bertrand, I., Bril, A., Gout, B., Debant, A., and Cambies, T. P. G. (2002) J. Cell Sci. 115, 629–640
47. Kato, S., Yamasaki, K., Mino, Y., and Tachi, T. (1994) J. Biol. Chem. 269, 3783–3789
48. Leinwand, L. A. (2001) Circ. Res. 89, 1880–1890
49. Wang, D., and Olson, E. N. (2001) EMBO J. 20, 2757–2767
50. Lu, J., McKinsey T. A., Zhang C. L., and Olson E. N. (2000) Mol. Cell 6, 233–244
51. Kamimura, T., and Yamauchi, M. (1993) J. Biol. Chem. 268, 18726–18733
52. Nakamura, T., and Yamauchi, M. (1993) J. Biol. Chem. 268, 18726–18733
53. Aikawa, R., Komuro, I., Yamazaki, T., and Yazaki, Y. (1992) Circ. Res. 71, 1529–1539
54. Chien, K. R. (2001) Nature 409, 245–246
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