Expression and function of COOH-terminal myosin heavy chain isoforms in mouse smooth muscle

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Submitted 9 November 2006; accepted in final form 19 March 2007

The contractile properties of different smooth muscles are highly variable, consistent with multiple functions. For example maximal shortening velocities can vary tenfold between different smooth muscles (11). Specific smooth muscle (SM) isoforms of the motor protein myosin may contribute to these unique contractile properties. Expression of these isoforms is developmentally regulated and altered in certain models of SM hypertrophy (10, 20, 30). The functional significance of isoforms of myosin heavy and light chains in mammalian SM has not yet been fully elucidated.

At least four isoforms of myosin heavy chain (MyHC) are expressed in SM, generated by differential splicing of a single gene at the 25K-50K junction in the NH2-terminal region of the myosin. The NH2-terminal splice site results in the insertion (SMb) of seven amino acids in the loop 1 region of the MyHC (27). At the COOH terminus, alternative splicing leads to the insertion of an exon coding for nine amino acids and a stop codon to form the isoform, SM2, with a nine amino acid nonhelical tail region, whereas the SM1 isoform, with no insert, has a longer nonhelical tail of 43 amino acids. The myosin isoforms are nonuniformly distributed among SM tissues. The SMb isoform predominates in phasic SMs, whereas SMa is the major isoform in tonic SMs (38).

Both of the COOH-terminal isoforms, SM1 and SM2, are present in all adult SM tissues although individual cells containing only SM1 or SM2 have been identified (22). The NH2-terminal insertion (SMb) is associated with an increased ATPase activity and actin velocity as determined by the in vitro motility assay (16) and a decreased affinity for ADP (9, 17, 34). Thus it is not unexpected that the absence of the SMb isoform results in a decreased unloaded shortening velocity in bladder from a SMb null mouse (15).

The functional role of the SM1 and SM2 isoforms is controversial. We have shown previously that estrogen treatment of ovariec-tomized rats increases the relative amount of SM1 in rat uterus, which was correlated with an increase in developed force and unloaded shortening velocity in permeabilized myometrial fibers (12). Hypertrophy of smooth muscle, following partial obstruction of the bladder, caused a decrease in both the COOH-terminal SM2 and NH2-terminal SMb isoforms (10, 21) that was associated with a decreased shortening velocity (30).

Variation in the expression of the SMa and SMb isoforms in conjunction with changes in SM1 and SM2 has, in part, contributed to the contradictory evidence in the literature. We developed transgenic (TG) mouse lines carrying a transgene for the MyHC isoforms SM1 or SM2, targeted to SM expression by the SM α-actin promoter (8, 23). SM1 transgene expression increased isometric force and shortening velocity whereas the SM2 transgene had the opposite effects. These results indicate that the COOH-terminal isoforms of SM myosin may play an important role in SM contractile function.

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MATERIALS AND METHODS

Animals. Mice used in these experiments were euthanized by exposure to ether or CO2 in accordance with the ethical treatment of animals using a protocol approved by the University of Illinois at Chicago Institutional Animal Care Committee or the University of Cincinnati Institutional Animal Care and Use Committee.

Construction of the SM-α-actin/SMαSM1 and SM-α-actin/SMαSM2 fusion genes. A 6.2-kb cDNA clone (SMHC-5), isolated from a rat stomach cDNA library (38) and identified as the SMαSM1 MyHC isoform was used. The SM2 sequence was generated by RT-PCR of rat stomach RNA using standard procedures and primers, containing a 5′ NcoI site and a 3′ NotI site, flanking the SM2 splice site. The RT-PCR product was subcloned into the T/A vector (Invitrogen) and sequenced. The NcoI-NotI fragment was excised and ligated into the SMHC-5 cDNA. An XhoI site was inserted 15 bp upstream of the MyHC ATG start site and a NotI site was inserted following the polyadenylation signal. We inserted a cMyc-tag or a V5-tag between the XhoI site and the ATG start site of the SM1 MyHC to identify the MyHC protein derived from the transgene. All PCR amplified cDNA fragments were subcloned and sequenced. Finally, the entire MyHC cDNA was ligated into the pGEM7Z vector containing the AatII (SV40) sequence forming the 10.3-kb SMP8-SM-α-actin promoter/MyHCCDNA/SM40 fusion gene (37). All boundaries were sequenced in the final construct.

Generation of TG mice. The fusion gene was digested by the restriction enzymes AarII and ClaI and separated by agarose gel electrophoresis. Following isolation and purification of the 10.3-kb construct, it was microinjected into pronuclei from fertilized eggs from CD-1 mice by Dr. Roberta Franks in the Transgenic Production Service, Research Resources Center at the University of Illinois at Chicago using a protocol approved by the University of Illinois at Chicago Animal Care Committee. Positive founders were identified by Southern blot analysis of PsI cut genomic DNA using an XhoI-SphI fragment from the 5′ region of the rat MyHC cDNA as a probe and by PCR analysis using the following primers: 5′-GATTAGCATTTGCTCATTCCGT and 3′-CTTGTATATGACGTATTTATG that span the MyHC-SV40 boundary. Heterozygous and wild-type progeny were routinely identified by PCR analysis of genomic DNA from tail samples using the above primers. In the experiments reported here, we have used two SM1 lines (F81 and F84) and three SM2 lines (F37, F63, and F67). We have not observed any significant differences in functional phenotype between lines for either transgene.

Transgene expression in mouse tissues. Total RNA was extracted from several SM and nonmuscle tissues using the procedure of Chomczynski and Sacchi (5). We used the following primers for analysis of the expression of the transgene mRNA in SM and non-SM tissues. The 3′ primer (AGGATGGCCACACGCAATATCT) and 5′ primer (ACCTGTAGGAGCTGATGCCAGATC) span the α-actin exon 2 and MyHC boundary. The PCR products were analyzed on agarose gels. For analysis of the relative levels of Smα and Smβ mRNAs, we used two primers (5′ ACCAGTTCCATCTGTGGCAC and 3′ CCATGTGAGACGTCGCT) spanning the area of divergence between Smα and Smβ MyHCs. These primers generated either a 500 or a 479-bp cDNA fragment corresponding to Smα and Smβ mRNAs, respectively, which were separated on 5% polyacrylamide gels. RT-PCR was performed using standard procedures with SuperScript RNase H− RT and Taq polymerase (GIBCO-BRL, Gaithersburg, MD). [32P]dCTP was included in the analysis of Smα and Smβ mRNAs.

SDS-PAGE. The SM1 and SM2 isoforms of myosin were separated by electrophoresis on large 6% acrylamide gels with 1% cross-linking by the Laemmli procedure (18). The gels were stained with Coomassie blue and the relative proportions of SM1 and SM2 isoforms present were determined by densitometry. We also ran tissue extracts from bladder on 10% polyacrylamide gels to determine the ratio of actin to MyHC in nontransgenic (NTG) and TG mice by densitometric analysis of Coomassie blue-stained gels. The data are expressed as means ± SE.

The essential light chains (LC17a and LC17b) of myosin were separated on urea-glycerol polyacrylamide gels (25, 31) and blotted onto nitrocellulose. Myosin LCs were identified using an antibody specific for smooth muscle LC20 (Cell Signaling Technology, antibody no. 3672) and a polyclonal antibody to smooth muscle light chains that we observed reacts with both LC20 and LC17 myosin light chains (Santa-Cruz Biotechnology, antibody no. sc-15370). The membranes were washed and incubated with horseradish peroxidase (HRP)-conjugated secondary antibody for 1 h at room temperature. After being washed, the membranes were developed with the use of enhanced chemiluminescence. The relative proportions of LC17b and LC17a in aorta and bladder tissue extracts from NTG and SM1 and SM2 TG mice were determined by densitometric analysis.

Western blot analysis. Protein expression from the transgene was examined in tissue extracts from bladder and aorta from mice carrying the cMyc-tagged SM1 or V5-tagged SM2 transgene and their NTG littermates. Proteins were separated by electrophoresis on 6% polyacrylamide gels with 1% cross-linking before electrophoretic transfer to PVDF membrane (36). cMyc expression was detected using a monoclonal antibody to cMyc protein (7) (Developmental Studies Hybridoma Bank, University of Iowa) and a secondary antibody (donkey anti-mouse IgG) conjugated to HRP. V5 expression was detected using a rabbit polyclonal antibody to the V5 peptide (Sigma, St. Louis, MO) and a goat anti-rabbit IgG conjugated to HRP. The blots were developed using the ECL kit from Amersham Biosciences (Piscataway, NJ). Antibodies were removed from the membrane in the presence of 2% SDS and 30 mM β-mercaptoethanol and the membrane was incubated with a polyclonal antibody, SM1Ab, or SM2Ab specific for either the SM1 or SM2 isoform of MyHC (14). SM1Ab and SM2Ab binding was detected using a HRP-conjugated goat anti-rabbit IgG and the Amersham ECL kit.

The relative ratio of α-actin to total actin in tissue extracts from TG and NTG bladders was determined using an antibody specific for SM α-actin (Sigma, St. Louis, MO) and an antibody (mAbC4) (19) that reacts with all isoforms of actin. The proteins in tissue extracts were separated by electrophoresis on 12.5% polyacrylamide gels and transferred to PVDF membrane (36). Binding of anti-actin antibodies was detected using a goat anti-mouse secondary antibody conjugated to hydrogen peroxidase and the ECL kit (Amersham). The membrane was exposed to X-ray film for different periods of time and the films were scanned using a Molecular Dynamics densitometer and analyzed using ImageQuant Software. The results are expressed as a ratio of SM α-actin pixels to total actin pixels for each protein concentration and each exposure. The means ± SE of these ratios in bladders from NTG and TG mice were compared using Student’s t-test.

Measurement of contractile function. Matched littermate mice between 8 and 14 wk old were euthanized by CO2 asphyxiation. Aortae or bladders were excised, placed in ice-cold physiological saline solution, and debrided of loose fat and connective tissue, and prepared for measurement of isometric force as previously described in detail (2, 33). Tissues were mounted isometrically and the length was adjusted to attain a passive force determined to place the smooth muscle in the range for optimal force generation. For the aorta, the applied tension was chosen to approximate that attributable to 100 mmHg. For the bladder, the applied tension for optimal force development was determined in preliminary experiments. The tissues were contracted in the presence of 80 mmol/l KCl for two contraction/relaxation cycles or until reproducible forces were obtained. KCl (10–80 mmol/l) concentration-dependent force relations were obtained in a cumulative fashion. Isometric force was normalized to the tissue cross sectional area. This area was calculated as the wet weight/length for the bladder or 2π wt/circumference for aorta. To quantitate tension generation kinetics, bladders were
stimulated with 80 mmol/l KCl. When a stable tension was achieved, tissues were rapidly (<0.5 s) shortened by 5% of their resting length. The time taken to redevelop half the maximum tension was used as an index of the rate of tension regeneration. Data were obtained with Acquire hardware and analyzed using AcqKnowledge Software (Biopac). Standard ANOVA was used as appropriate; differences were accepted as statistically significant for P ≤ 0.01.

RESULTS

Generation of TG mice. The components of the SMP8 α-actin promoter/MyHC construct are shown in Fig. 1A. We identified 5 SM1 founder mice of the 36 analyzed and 7 SM2 founder mice out of 52 analyzed by Southern blot and PCR analysis as illustrated in Fig. 1, B and C. In Fig. 1B, number F86 had a very low copy number compared with numbers F81 and F84. In all cases, the transgene was transmitted to the F1 generation. The tissue distribution of the SM2 (F37) transgene mRNA is shown in Fig. 1C. mRNA from the SM2 transgene is expressed in SM tissues such as aorta, mesenteric artery, bladder, stomach, and uterus. It is not present in the heart, brain, or kidney tissue from TG mice. This is a typical tissue distribution for transgenes regulated by the smooth muscle specific SMP8 promoter (8, 37).

Transgene expression. The presence of myosin heavy chain protein derived from the transgene was detected in SM tissue using antibodies to cMyc or V5 tags as shown in Fig. 2A. There was no expression of cMyc or V5 in SM from NTG littermates. In SM1 and SM2 TG lines without the cMyc and V5 tags, we identified expression of MyHC mRNA from the transgene in certain SM tissues that express primarily the NH2-terminal SMb isoform. The transgenes contain only the SMa isoform of MyHC. Therefore, the increased expression of SMa mRNA in certain tissues from TG mice can demonstrate that the transgene is indeed expressed. RT-PCR was used to assess the relative proportions of SMa and SMb mRNAs as shown in Fig. 2B. There is a significant increase in SMa mRNA expression in bladder and mesenteric artery from TG mice compared with NTG controls. The level of SMb mRNA appears unchanged in bladder and mesenteric artery but is decreased in the caudal artery.

Thus the expression of SM MyHC mRNA derived from the SM1 and SM2 transgenes (Fig. 2B) and the presence of significant levels of cMyc-MyHC and V5-MyHC protein derived from the transgene (Fig. 2A) suggests that the presence of the transgene should have a significant impact on the SM1: SM2 protein ratio in SM tissues. SM1 and SM2 MyHC isoforms were separated on low cross-linking gels and examples of this separation in homogenates of bladder tissue from TG and NTG mice are shown in Fig. 3. Interestingly, there was no significant difference in the percent SM1 expressed in aortae from NTG (56.1 ± 2.4%, n = 10), SM1 TG (51.2 ± 4.6%, n = 5), and SM2 TG (57.5 ± 2.6%, n = 6) or bladders

![Fig. 1. Generation of mice carrying a transgene for smooth muscle (SM) MYOSIN isoforms.](image)

AJP-Cell Physiol • VOL 293 • JULY 2007 • www.ajpcell.org
from NTG (55.4 ± 0.6%, n = 10), SM1 TG (54.7 ± 3.5%, n = 6), and SM2 TG (55.2 ± 3.6%, n = 5) mice. On the basis of a pooled estimate of the variance, changes as large as 13% would not be significant at the P = 0.05 level. In addition, there was no significant increase in total MyHC expressed in the TG animals based on measurements of the ratio of actin to MyHC in bladder from TG mice (SM1 = 1.20 ± 0.03, n = 4; SM2 = 1.21 ± 0.10, n = 4) compared with NTG (1.19 ± 0.04, n = 8) control mice. Neither did we detect any significant change in the pattern of proteins separated by SDS-PAGE between NTG and TG samples as shown in Fig. 4. The nonmuscle myosin (NMM) present in the smooth muscle tissues from SM1 (F84) and SM2 (F63) TG mice. Primers on either side of the NH2-terminal splice site for SMa and SMb MyHC isoforms were used to generate cDNA from total RNA isolated from the smooth muscle tissues. These primers were also used to amplify the cDNA generating two bands, of 500 bp and 479 bp, corresponding to the SMb and SMa isoforms of MyHC, respectively. The transgene corresponds to the SMa isoform. Therefore increased expression of SMa mRNA in tissues that normally predominantly express SMb mRNA indicates expression of mRNA from the transgene. Note the shift from SMb to SMa in TG relative to NTG tissues.
muscle tissue is separated with the SM1 and SM2 isoforms on low cross-linking gels and runs below the SM2 isoform in Fig. 3. There is very little NMM present in total extracts of bladder tissue and one can see only a trace in the gels presented in Fig. 3. In addition, the expression of NMM was unchanged in tissues from TG animals.

We also examined the relative amounts of LC17b and LC17a in aortae and bladders from TG and NTG tissues. We found no statistically significant difference in the ratios of LC17b to LC17a in TG (SM1, 0.64; SM2, 0.60) compared with NTG (SM1, 0.54; SM2, 0.47) in aortae tissues; n = 4 in all groups and P = 0.61 for SM1 and P = 0.26 for SM2. We did not detect any LC17b in bladder tissue in any mouse line.

We also found no significant alteration in the expression of SM α-actin relative to total actin in bladder from TG (SM1 = 1.18 ± 0.03, n = 4; SM2 = 1.71 ± 0.08, n = 4) compared with NTG (1.39 ± 0.16, n = 8) mice.

Smooth muscle function. Figure 5 shows the force-KCl concentration relation for bladder and aorta in mice expressing the SM1 or SM2 transgene. Results from the F81 line of SM1 TG mice and the F67 line of SM2 TG mice are shown. Similar results were observed in additional lines of mice carrying the transgene for each COOH-terminal MyHC isoform. The contractile parameters from these experiments are summarized in Table 1. Maximum force was significantly (P < 0.01) different from NTG control mice in both aorta and bladder from SM1 and SM2 TG mice. The ED50 values of the KCl concentration did not differ significantly between NTG and TG bladders or aortae. Figure 5, A and B, show the responses of the SM1 mouse aortae and bladders respectively to KCl activation. Tissues from SM1 TG mice showed a consistently higher
maximum force in response to KCl than tissues from NTG littersmates. Maximum tension development in aortae and bladder from SM1 TG mice was elevated by 72% and 92%, respectively, compared with NTG tissues.

In contrast, aortae and bladders from SM2 TG mice had a consistently lower maximum force in response to KCl. Figure 5, C and D, illustrate the response of the TG aortae (57 ± 14% of NTG) and TG bladders (80 ± 3% of NTG), respectively. These trends were observed in a second TG line for both the SM1 and SM2 transgene, suggesting that these functional differences are due to expression of the SM1 or SM2 transgene and are not the result of the position of the insertion of the transgene.

The differences in maximal force generation (SM1>NTG>SM2) were unlikely due to differences in activation. We tested this possibility by using β-escin to permeabilize bladders in a high Ca²⁺-containing solution to maximize intracellular [Ca²⁺]. In all cases, isometric force increased slightly (<15%) above the preexisting force in KCl. Importantly the percent increases did not differ between TG and NTG for either transgene (n = 3 for SM1 pair; n = 4 for SM2 pair). Thus the differences in maximum force among TG and NTG animals were maintained, under conditions selected to maximize activation.

**Contraction kinetics.** We assessed contractile kinetics by measuring the rate at which the bladder was able to regenerate tension following a rapid (0.5 s) stepwise shortening of 5% of the resting length. As shown in Fig. 6 and Table 1, SM1 TG bladders exhibited a significantly faster rate of tension regeneration compared with NTG. In contrast, the SM2 bladders showed the opposite response, having a significantly slower rate of tension regeneration than NTG.

**DISCUSSION**

We present evidence for distinct functional phenotypes in smooth muscle associated with the SM1 and SM2 MyHC isoforms. The phenotypes associated with transgenes encoding these COOH-terminal MyHC isoforms are present in multiple lines from each transgene indicating that alterations in contractile function are not due to artifacts of the position of transgene insertions. Mice containing the SM1 transgene have increased maximal tension and rate of tension redevelopment, which are opposite to the changes observed in tissues from mice carrying the SM2 transgene. This suggests that functional changes in smooth muscle tissues from our TG mice result from expression of the transgene itself.

We demonstrated that cMyc- and V5-labeled MyHC are expressed from the transgene in bladder and aorta (Fig. 2A). Further supporting the activity of the transgene is the shift from SMb to SMA mRNA (Fig. 2B). Intriguingly, relatively little demonstrable change was observed in the ratio of SM1:SM2 protein in smooth muscle tissues from heterozygous SM1 or SM2 TG mice using SDS-PAGE. This is an important finding as it suggests that the ratio of the COOH-terminal isoforms of MyHC is tightly regulated in smooth muscle. This idea is also consistent with the difficulty in developing experimental models that alter the isoform ratio. It is possible to alter the SM1:SM2 ratio using experimental interventions such as estrogen administration to ovariectomized rats (12), and partial obstruction of the bladder (10, 30). However, the shifts in the SM1:SM2 ratio in these experimental models are not very large (<10 percentage points) and are substantially exceeded by the associated functional changes. Conservation of the SM1:SM2 ratio may be compared with the maintenance of the stoichiometry between myofibrillar proteins in the heart. Myofibrillar proteins, overexpressed in the heart from a transgene, replace to a greater or lesser extent the endogenous protein, while maintaining the stoichiometry of the myofibrils (28). In our TG mice, we also see significant changes in force and kinetics associated with expression of the SM1 or SM2 transgene accompanied by only relatively small changes in the SM1:SM2 ratio. This is consistent with previous studies showing that relatively small changes in myosin isoform ratios can be

**Table 1. Functional properties of aortae and bladder from SM1 and SM2 TG and NTG littermates**

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<tr>
<th>Aorta %Maximum Force</th>
<th>NTG</th>
<th>TG</th>
</tr>
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<tbody>
<tr>
<td>SM1 F81 Mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ED₉₀, mmol/l</td>
<td>26.8±3.5</td>
<td>19±2.1</td>
</tr>
<tr>
<td>%Maximum Force</td>
<td>100</td>
<td>192±11*</td>
</tr>
<tr>
<td>Recovery 1/2,s</td>
<td>4.2±0.5</td>
<td>4.4±0.4</td>
</tr>
<tr>
<td>SM2 F67 Mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ED₉₀, mmol/l</td>
<td>51.9±5.3</td>
<td>38.7±1.1</td>
</tr>
<tr>
<td>%Maximum Force</td>
<td>100</td>
<td>80±3*</td>
</tr>
<tr>
<td>Recovery 1/2,s</td>
<td>4.2±0.5</td>
<td>4.4±0.4</td>
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<table>
<thead>
<tr>
<th>Bladder ED₅₀, mmol/l</th>
<th>NTG</th>
<th>TG</th>
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<tbody>
<tr>
<td>SM1 F81 Mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%Maximum Force</td>
<td>172±14*</td>
<td>100</td>
</tr>
<tr>
<td>Recovery 1/2,s</td>
<td>4.2±0.5</td>
<td>4.4±0.4</td>
</tr>
<tr>
<td>SM2 F67 Mice</td>
<td></td>
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</tr>
<tr>
<td>%Maximum Force</td>
<td>19±2.1</td>
<td>57±1*</td>
</tr>
<tr>
<td>Recovery 1/2,s</td>
<td>4.2±0.5</td>
<td>4.4±0.4</td>
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Values are means ± SE; n = 4–5 animals. TG, transgenic genotype; NTG, nontransgenic genotype; SM, smooth muscle. *P ≤ 0.01.
associated with significant alterations in smooth muscle (12, 30) as well as cardiac (35) contractile function.

There are several hypotheses by which changes in MyHC isoform population can lead to major alteration of functional properties. Babu et al. (3) found altered expression of thin filament regulatory proteins associated with a change from the SMb to SMa NH2-terminal MyHC isoform. Since we found opposite functional effects for the SM1 and SM2 animals, we think that the effects of these proteins, which may have been altered with the transgene expression, are unlikely. We found no detectable LC17b in bladder tissue extracts and no differences between TG and NTG tissues for either SM1 or SM2 lines. A small (~10%) and not statistically significant increase in the ratio of LC17b to LC17a in aorta was observed in both SM1 and SM2 TG lines. Thus, it is unlikely that these increases could contribute to the opposite functional effects we observe in SM1 and SM2 TG tissues.

Changes in the SMa MyHC isoform have been reported to alter contractile characteristics in the bladder (6, 32). Babu et al. (2) have also shown that elimination of the SMb MyHC isoform in bladder leads to a significant decrease in maximal tension development and shortening velocity in the presence of unchanged ratios of SM1:SM2. In contrast, we find an increase in SMa mRNA expression in bladders from both SM1 and SM2 TG mice. However, we found significant and opposite changes in maximal tension development and velocity of shortening. Thus increased expression of SMaSM1 or changes in maximal tension development and velocity of shortening. Thus increased expression of SMaSM1 or SMaSM2 appears to alter contractile function independently of NH2-terminal isoform expression in the aorta, which contains principally the SMa isoform, similar changes in maximum force generation to those seen in the TG bladder further support our hypothesis that these contractility changes are associated with altered COOH-terminal isoform expression.

The functional role of the COOH-terminal SM1 and SM2 MyHC isoforms is not known with certainty. The nonhelical tail regions of nonmuscle MyHCs (1, 13) have been implicated in modulation of ATPase activity and thick filament assembly. We have shown that removal of the SM1 COOH-terminal region affects the NH2-terminal Mg-ATPase activity of phosphorylated myosin in vitro (14). It is also possible that the SM1 and SM2 isoforms assemble into thick filaments with different stabilities and functional properties (26, 29). In support of this hypothesis, paracrystals formed in vitro from SM1 or SM2 rod regions have different molecular packing characteristics and stability (26, 29). The greater stability of the SM1 rod thick filaments (29) may result from interaction of the SM1 COOH-terminal tail with an adjacent myosin molecule (4).

Our hypothesis is that overproduction of the SM1 or SM2 isoform from multiple copies of a transgene may change the dynamics of filament assembly, producing a different population of thick filament types with only minor changes in the proportions of SM1 and SM2 expressed in the tissue. Recent evidence (29) suggests that homodimers of SM1 and SM2 heavy chains are preferentially formed in vivo, whereas significant heterodimeric formation is seen in vitro. Whether thick filaments in vivo contain both SM1 and SM2 MyHC homodimers is unknown. However, isolated smooth muscle cells containing only SM1 or SM2 have been identified, in addition to cells containing both isoforms (22), therefore, filaments containing only SM1 or SM2 can be formed.

An example of how small differences in the SM1:SM2 isoform ratio can lead to large differences in filament types is illustrated in Fig. 7, A and B. If one assumes that a 50:50 mix of SM1 and SM2 MyHC chains are synthesized from the endogenous MyHC gene and that these SM1 and SM2 MyHCs randomly assemble into thick filaments, then the expected result would be the formation of 50% hybrid filaments consisting of SM1 and SM2 homodimers and 25% each SM1 only filaments or SM2 only filaments (Fig. 7A). An overall 8% increase in tissue expression of SM1 can result in a doubling of filaments containing only the SM1 isoform relative to those containing only the SM2 (Fig. 7B). In this way, a small change in SM1:SM2 ratio may be amplified by its effect on filament assembly.

Our data show that the COOH-terminal regions of the MyHC isoforms, though not spatially located close to the myosin ATPase site, are associated with distinct and significant differences in contractility. Our TG mouse model has enabled us to clearly demonstrate that the COOH-terminal MyHC isoforms can significantly affect tension development and kinetics of force redevelopment in smooth muscle. Importantly, our evidence indicates that the COOH-terminal isoform distribution is a well-controlled property of smooth muscle. The existence of functionally different COOH-terminal myosin isoforms in smooth muscle cells provides an additional mechanism for modification and fine tuning of contractile properties in different smooth muscle tissues, and thus may provide a target for therapeutic interventions.

Fig. 7. Schematic showing projected filament assembly from different populations of SM1 and SM2 homodimers. This model illustrates how a relatively small shift in SM1 (~8%) can elicit a major shift in filament composition and potentially underlie the significant change in contractility.
ACKNOWLEDGMENTS
The monoclonal antibody to cMyc (developed by G. I. Evan, G. K. Lewis, G. Ramsey, and J. M. Bishop) was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biological Sciences, Iowa City, IA.

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