Myosin II isoforms in smooth muscle: heterogeneity and function

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Eddinger TJ and Meer DP. Myosin II isoforms in smooth muscle: heterogeneity and function. Am J Physiol Cell Physiol 293: C493–C508, 2007. First published May 2, 2007; doi:10.1152/ajpcell.00131.2007.—Both smooth muscle (SM) and nonmuscle class II myosin molecules are expressed in SM tissues comprising hollow organ systems. Individual SM cells may express one or more of multiple myosin II isoforms that differ in myosin heavy chain (MHC) and myosin light chain (MLC) subunits. Although much has been learned, the expression profiles, organization within contractile filaments, localization within cells, and precise roles in various contractile functions of these different myosins are not well understood. However, data supporting unique physiological roles for certain isoforms continue to build. Isoform differences located in the S1 head region of the MHC can alter actin binding and rates of ATP hydrolysis. Differences located in the MHC tail can alter the formation, stability, and size of the myosin thick filament. In these distinct ways, both head and tail isoform differences can alter force generation and muscle shortening velocities. The MLCs that are associated with the lever arm of the S1 head can affect the flexibility and range of motion of this domain and possibly the motion of the S2 and motor domains. Phosphorylation of MLC20 has been associated with conformational changes in the S1 and/or S2 fragments regulating enzymatic activity of the entire myosin molecule. A challenge for the future will be delineation of the physiological significance of the heterogeneous expression of these isoforms in developmental, tissue-specific, and species-specific patterns and or the intra- and intercellular heterogeneity of myosin isoform expression in SM cells of a given organ.

nonmuscle myosin; expression

MYOSIN is a protein defined by its ability to bind with actin and hydrolyze ATP. These molecular motors include over 20 classes of structurally and functionally distinct molecules and are involved in an ever-increasing number of identified intracellular and intercellular functions (78). In this review, we will focus on the class II MHCs that are known to be present in smooth muscle (SM), specifically, SM and nonmuscle (NM) myosin molecules. As it is not possible to cover everything related to these proteins, we will focus on their isoforms, organization, expression, distribution, and what is known about their function. Because of the extensive literature on this topic, we can only cite and comment on a limited number of relevant articles.

Myosin molecules of the myosin II family are composed of six subunits, two myosin heavy chains (MHCs) and two pairs of myosin light chains (MLCs), one of which associates with each of the MHC heads. The MHCs have two anatomically distinct domains, including the globular subfragment one (S1) head and α-helical rod domain (Fig. 1). The S1 head is composed of the motor domain (the site of actin binding and ATP hydrolysis) and the lever arm or neck domain (the site of MLC binding). The movement of the lever arm relative to the motor domain (in addition to other changes) results from movements at the junction of these two domains. This hinge-like region is referred to as the converter domain (Fig. 1). The α-helical MHC rod pairs with a second MHC rod to form a coiled-coil tail domain that includes the site(s) of myosin molecule association for filament formation (130). The 28-amino acid repeat typical of an α-helical coiled-coil (97, 114) does not extend through the S2 hinge region in rat and rabbit myosin (93), suggesting that the stability of this region is lower than the rest of the coiled-coil. Conformational changes in the S2-light meromyosin junction (LMM) (as suggested by susceptibility to enzymatic digestion) are reported to be coupled to cross-bridge cycling (154, 155), and Lauzon et al. (84) reported that instability of the head-S2 junction (unwinding of the coiled-coil?) is required for optimal SM myosin mechanical performance. Thus, in addition to the changes that occur in the S1 head, it may be necessary for both ends of the S2 fragment of the rod to “unfold” or bend for proper cross-bridge cycling.

SM cells (SMCs) express multiple isoforms of class II myosins, including both SM and NM isoforms. In SM, there is a single SM MHC gene that codes for four unique MHC molecules via two alternate splice sites (Fig. 2). Alternate splicing of a 21-nt exon at the 5′ end of the gene results in the presence or absence of a seven-amino acid insert at the 25- to 50-kDa junction in the S1 head region (Figs. 1, loop 1, and 2) of the molecule (referred to as SMB and SMA, respectively; Fig. 3) (53, 75, 161). An alternate splice of a 39-nt exon in the nonhelical portion of the 3′ end of the gene codes for 9 unique amino acids at the COOH terminus and an in-frame stop codon that results in a molecule that is 34 amino acids shorter (SM2) than the alternately spliced isoform (SM1) (7, 109, 110) (Figs. 1, tail insert; 2, splicing; and 3). SM myosin with the head insert and without the tail insert (SM1B) is 1,979 amino acids

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long, whereas the SM MHC without the head insert and with the tail insert (SM2-SMA) is 1,938 amino acids long. There are two SM MLC17 isoforms that also result from the alternate splicing of a single SM MLC gene (82, 87, 108). The two isoforms are the same size (151 amino acids) but differ in five of the last nine COOH amino acids. The more acidic protein is MLC17a, and the more basic protein is MLC 17b (a clarification of the nomenclature used for these isoforms can be found in Ref. 33). The SM MLC20 subunit can also be expressed as two different isoforms, which are the products of two different genes (79, 150). The two MLC20 isoforms are also the same size (171 amino acids) but differ by 11 residues with the more basic isoform being the SM isoform and the more acidic isoform being the NM isoform (44). Each myosin S1 neck domain binds one MLC20 and one MLC 17.

There are also at least three NM class II MHCs that can be expressed in SM tissues (Fig. 3). The NM MHC isoforms result from alternative splicing of multiple genes (NM MHC-A, NM MHC-B, and NM MHC-C; Fig. 3) (50, 70, 136, 137) and multiple splice sites on at least one of the genes (NM MHC-B) (67, 149). The NMA and NMB MHC isoforms are similar in size to the SM MHC isoform without the head or tail insert (NMA is 1,960 amino acids long and NMB is 1,976 amino acids long). The NMC isoform has an additional 20 amino acids at the NH2 terminus and shows significantly more variability in amino acid sequence from the SM MHC than the other two NM MHCs (1,995 amino acids; Fig. 3).

**MHC and MLC Associations**

The arrangement of the MHC isoforms in a given myosin molecule appears to be variable based on reports in the literature. Kelly et al. (74) used SM isoform-specific antibodies with affinity chromatography to show that only SM MHC homodimers (tail isoforms, SM1 and SM2) exist in the bovine aorta (Fig. 2). In contrast, we (152) reported that there are homodimers and heterodimers of the SM MHC tail isoforms present in swine SM tissues (the stomach, uterus, an aorta) (Fig. 2). The presence or absence of the MHC head isoforms SMA/B does not appear to be correlated with expression of a specific MHC tail isoform as there are reports of high expression levels of either SMA or SMB in tissues with similar SM1/2 MHC content (34, 75). Kelly et al. (73) reported that NM MHC-A and -B isoforms only form homodimers that
SM and NM MLC\textsubscript{20} isoforms can be expressed in SMCs, and it is currently not known if and how these MLC isotypes associate in vivo with SM and NM MHCs. In swine carotid media, the level of NM MHC protein expressed (∼14%) matches the level of NM MLC\textsubscript{20} protein (∼16%), suggesting that the NM and SM isoforms are not promiscuous. However, in platelets, where there is only NM MHC protein (primarily A) expressed, the SM-to-NM MLC\textsubscript{20} protein expression ratio is 50:50 (44). This suggests that NM and SM MHC and MLC isoforms can associate to form distinct myosin molecules. In primary cultures of rat thoracic aortic SMCs, it has been reported that the NM MLC\textsubscript{20} isoform content is inversely related with cell growth, suggesting higher content in mitotic cells (101). However, no difference was observed in the amount of phosphorylation of the NM versus SM MLC\textsubscript{20} isoforms in relaxed or activated cells, as would be predicted if these isoforms were individually regulated for unique cellular functions (101).

The two MLC\textsubscript{17} isoforms are uniquely expressed in various SM organs and have been reported to range from at or close to 100% MLC\textsubscript{17a} in tissues such as the chicken gizzard and the mammalian stomach, intestine, and trachea (58, 75, 94) and to >50% MLC\textsubscript{17b} in tissues such as the uterus and aorta (19, 57, 58, 94). The possibility of a functional significance for this variability is controversial and is addressed below (see the Role for MLC\textsubscript{17a/b} and MLC\textsubscript{20} isoforms). We (134) have reported that there is no correlation in the degree of expression of SM1/2 MHC and MLC\textsubscript{17a/b} isoforms in freshly isolated rabbit arterial SMCs. It is unclear if there is any strong correlation between MLC\textsubscript{17a/b} isoform expression and SMA/B MHC isoform expression.

Myosin Isoform Expression in SM

Unlike striated muscle, SM was believed to express only one type of myosin until 1975, when Burridge and Bray (15) reported the possibility of two MHCs in the chicken gizzard. Another decade passed before another full paper (125) reported the presence of SM MHC isoforms in various SM tissues with the hypothesis of unique functions for each isoform. The size difference between the two SM MHC isoforms could be explained by a difference in size of the LMM fragment (38). These studies were followed quickly by molecular studies showing a 3- to 5-kDa difference located at the COOH terminal of the molecule. Subsequent work in which the entire gene was sequenced showed there was a second alternate splice site in the motor domain (see above for references). In the mid-1980s, the presence of NM MHC in SM was first reported in cultured SMCs (71, 124) and then shown to be in SM tissues (44). This suggests that NM and SM isoforms are not promiscuous. However, there are insufficient data to warrant a consensus opinion on the molecular arrangements in SMCs of MHC isotypes. The identification and understanding of the possible unique regulation and functional roles for these subunits and molecular pairings will require that we determine how and when these subunits are expressed, organized (molecules and filaments), and distributed in cells and tissues.
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MAQKQQLSDLDEKFLPVDKFINPSVQAQDAWALKLVWPSKEQGFBAASIKEEKGDEVVVLEROVGBKKTVDKIDQCMKNPPKFSK
MAQKQQLSDLDEKFLPVDKFINPSVQAQDAWALKLVWPSKEQGFBAASIKEEKGDEVVVLEROVGBKKTVDKIDQCMKNPPKFSK
MAQKQQLSDLDEKFLPVDKFINPSVQAQDAWALKLVWPSKEQGFBAASIKEEKGDEVVVLEROVGBKKTVDKIDQCMKNPPKFSK
MAQKQQLSDLDEKFLPVDKFINPSVQAQDAWALKLVWPSKEQGFBAASIKEEKGDEVVVLEROVGBKKTVDKIDQCMKNPPKFSK
MAQKQQLSDLDEKFLPVDKFINPSVQAQDAWALKLVWPSKEQGFBAASIKEEKGDEVVVLEROVGBKKTVDKIDQCMKNPPKFSK
MAQKQQLSDLDEKFLPVDKFINPSVQAQDAWALKLVWPSKEQGFBAASIKEEKGDEVVVLEROVGBKKTVDKIDQCMKNPPKFSK
MAQKQQLSDLDEKFLPVDKFINPSVQAQDAWALKLVWPSKEQGFBAASIKEEKGDEVVVLEROVGBKKTVDKIDQCMKNPPKFSK

HAMAECLCNRAEVLHUNERYSGLIYYTSOLFPCVNVYPKYULHISYEIKVMDYOKKHREMPHPHYAIADTAYRSMQLQORDESQILCTORSOAQCTENTKTVIQY
HAMAECLCNRAEVLHUNERYSGLIYYTSOLFPCVNVYPKYULHISYEIKVMDYOKKHREMPHPHYAIADTAYRSMQLQORDESQILCTORSOAQCTENTKTVIQY
HAMAECLCNRAEVLHUNERYSGLIYYTSOLFPCVNVYPKYULHISYEIKVMDYOKKHREMPHPHYAIADTAYRSMQLQORDESQILCTORSOAQCTENTKTVIQY
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HAMAECLCNRAEVLHUNERYSGLIYYTSOLFPCVNVYPKYULHISYEIKVMDYOKKHREMPHPHYAIADTAYRSMQLQORDESQILCTORSOAQCTENTKTVIQY
HAMAECLCNRAEVLHUNERYSGLIYYTSOLFPCVNVYPKYULHISYEIKVMDYOKKHREMPHPHYAIADTAYRSMQLQORDESQILCTORSOAQCTENTKTVIQY

Fig. 3—Continued

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Fig. 3—Continued

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Putative Converter Domain

IQ Motif

IQ Motif
with the use of biochemical and immunochemical techniques and paralleled their initial identification. More recently, sequencing of various genomes has lead to the identification of additional myosin isoforms based on sequence homology. Evidence for unique regulation and function has not been as straightforward, with seemingly contradictory results for much of what has been reported.

Developmental Regulation of Myosin Expression

SM and NM myosin isoform expression is developmentally regulated in all SM tissues examined. Unfortunately, the fact that this regulation is organ and species specific has not always been taken into account since the mid- to late 1980s, when it was first reported, and thus remains a complicating factor for many studies that use tissues from different aged animals and species. The first reports to suggest developmental regulation were from tissue culture experiments showing that SM and NM MHCs change quantitatively with cell cycle (6, 71, 124). Developmental studies showed that there is a larger amount of NM myosin in SM tissues from embryonic and neonatal animals than there is in adults. In some species and tissues, the NM myosin content is relatively high in embryos and neonates (approaching 50% of total myosin) and decreases to ~10% as the animal becomes sexually mature (swine, rat, mouse, and bovine) (37, 40, 43, 44). In the rabbit, NM myosin expression is high in SM tissues in the embryo but is not present in the neonate or adult SM tissue (12, 54, 81). The decrease in NM MHC expression in SM tissues with development correlates with an increase in expression of either SM1 (rat aorta) or SM2 (mouse aorta and swine tissues) (37, 40). That is, NM MHC is replaced by SM MHC as the animal matures. Assuming unique functions for SM and NM myosins, the reduction from a

Fig. 3. Sequence alignment of the four SM MHC and three NM MHC isoforms. The accession numbers of the myosins are as follows: human SMB-SM1 (NP_001035203); human SMA-SM2 (NP_074035); human NMA (P35579); human NMB (P35580); and human NMC (Q7Z406). Major putative functional domains are noted by the underlines. With the exception of the two alternative splice differences, the SM MHC isoforms are identical (top two sequences). The SM MHC sequence difference resulting in additional AAs from the NH2-terminal alternative splice are located at the opening to the nucleotide binding pocket (loop 1) and the sequence difference/addition resulting in AAs from the COOH-terminal alternative splice are located at the nonhelical tail of the MHC (shown in bold). NMA and NMB MHCs have lengths that are between the longest and shortest SM MHCs, while NMC MHC is significantly longer than the other SM and NM MHC isoforms, primarily due to an additional 20 NH2-terminal AAs. Boxed red prolines (P) are beginning and end of putative coiled-coil domain.

RED - Differences between SM and NM
PINK - Differences between SM, NMA and NMB
BLUE - Differences between SM, NMA, and NMB

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significant percentage of NM myosin in embryonic and neonatal SM tissue down to ~10% in the adult (0% in mature rabbit SM tissues) suggests a change in primary cell function with differentiation, such as a loss in the capacity for hyperplasia and gain in capacity for tissue shortening and force development. Studies have also reported an increase in the SMB MHC in rat lung SM tissue with development (90–92, 162).

**Tissue-Specific and Cell Expression-Specific Myosin Heterogeneity**

Different SM tissues have unique physiological properties, and one classification scheme developed in the late 1960s that is still generally used designates SM “types” as phasic and tonic (144). Others have proposed that further distinctions are necessary. Golebofen (49) suggested that there are three “dimensions” that can be used to classify smooth muscles including 1) degree of automaticity from fully myogenic to complete nervous control, 2) quality of automaticity with rates of spike potentials in the msec range to the hour rhythm of slow waves, and 3) phasic versus tonic with phasic activity masking tonic activity in some tissues (portal vein and uterus). These distinctions may prove important in trying to identify functional significance for the myosin isoforms within and among different SM tissues. Biochemical and immunological methods have been used to determine possible correlations between SM tissue physiology and myosin isoform expression. The SM MHC tail isoforms (SM1 and SM2) show differences in levels of expression that seem to lie in a fairly narrow range between SM tissues (typically between 40:60 and 60:40 SM1 to SM2). Rovner and Murphy (125) reported an ~50:50 ratio of SM1 to SM2 for all SM tissues they examined. Although most studies support similar ratios, others report ratios different than 50:50 (Ref. 22; see below) and report that the ratio can change with the stage of development (see above).

Myosin heterogeneity has been identified in tissues such as bovine vascular SM and the opossum esophagus (41–43, 105, 106, 148). There are reports of SMCs in the developing bovine aorta that express only fetal (NMB?) myosin (166, 167). Frid et al. (43) and Giuriato et al. (47) reported a gradient of NM versus SM myosin expression as a function of distance from the arterial lumen in bovine and rabbit arteries. Holifield et al. (65) reported two populations of vascular SMCs in a gradient from lumen to adventitia. One of these cell populations was reported to contain SM proteins and the cells did not spread in culture, whereas the other cell population contained no SM myosin and proliferated in culture. Lau and Chacko (83) have also reported two types of SMs in the rabbit bladder that expressed SM and NM myosin. One type forms long cells that can undergo cell division, whereas the other forms short cells expressing α-actin in culture. Chiavegato et al. (22) performed an immunological screen of a variety of bovine tissues and reported that the SM1/2 MHC isoforms are unequally distributed. In some organs, the distribution of myosin isoforms is layer dependent. For example, it has been reported that while SM1 MHC is expressed in both the longitudinal and circular layer of the rabbit myometrium, SM2 MHC is not present in the circular layer (18).

The SMA/B MHC isoforms appear to have a much wider range of expression levels than the SM1/SM2 MHC isoforms. SMB MHC is the primary isoform expressed in tissues like the bladder, saphenous, and stomach antrum, whereas SMA MHC is the primary isoform expressed in tissues like elastic arteries, the stomach fundus, and the uterus (34, 54, 115, 161). We examined SMA/B MHCs isoform distribution in the stomach and found that they were uniquely distributed between the regions of this organ and between neighboring cells in a given region (Fig. 4). Isolated cells from the stomach also showed regional cellular heterogeneous expression of myosin isoforms (Fig. 5). In general, slow tonic contractions were localized to the fundus of the stomach, and fast phasic contractions were localized to the antrum (129). SMB MHC immunoreactivity was observed to increase as one follows the greater curvature of the stomach from the tonic fundus to the phasic antrum. In addition, in any given region of the stomach, cells showing high SMB MHC immunoreactivity were equally likely or not to be next to a cell with low SMB MHC immunoreactivity (115). This might suggest that if there are unique functional roles for these isoforms they work at the single cell level and that function of the tissue is not simply a population of homogeneous cells all doing the same thing at the same time. Taken to another level, within a given SMC, the distribution of a given myosin isoform can also be uniquely distributed. In stomach antrum SMCs, the SMB MHC isoforms have been observed to be distributed with an increased presence at the cell’s periphery (Fig. 6). This arrangement would allow for unique regulation of these specific myosin isoforms via their spatial separation and possible unique function within the SMC.

mRNA for the NMA and NMB MHC isoforms and these proteins have been reported to be present in fairly high levels in many SM tissues (16, 50, 117), while NMC MHC expression is low in SM organs (but high in nervous tissue) (50). The expression of NMB MHC is also variable in SM tissue (Fig. 7). Based on changes in SM versus NM MHC expression in nonconfluent versus confluent cultured SMCs (71, 124), one might hypothesize that the cells with the higher NM MHC immunofluorescence are cells that are “dedifferentiated” and perhaps mitotically active. Further work is needed to determine if and how the cells expressing significantly more NM MHC differ physiologically from their neighbors expressing little NM MHC.

MLC$_{17a}$ isoforms are also expressed at different levels in different tissues ranging from more than ~80% MLC$_{17a}$ in tissues like the carotid, pulmonary, and saphenous arteries, bladder, trachea, stomach, and intestines to levels closer to 50% in the aorta, renal, and femoral arteries and uterus (19, 29, 34, 54, 57, 58, 94). We (33) also reported cellular variability in MLC$_{17a}$ expression from ~50–90% in freshly isolated SMCs from the rabbit carotid and aorta. Thus, these isoforms also show dramatic cellular and tissue heterogeneity. MLC$_{20}$ isoforms are also differentially expressed in different tissues and with growth state in cultured SMCs (31, 44, 101, 104). At this time, there is no convincing evidence for unique functional roles for the isoforms of either of these light chains (see Role for MLC$_{17a}$ and MLC$_{20}$ isoforms).

**Possible Functional Role for Cellular Heterogeneity**

There is no known functional role for heterogeneity of myosin isoform expression within a given organ. Unlike stri-
ated muscle, where fibers expressing different myosin isoforms are known to be grouped into motor units that can be recruited uniquely by their respective motor neuron, there are no direct data suggesting analogous motor units in SM tissues. SMCs are thought to function as a syncitium, and, because they pull on each other rather than on a skeleton, it seems illogical that they would not all contract at the same time or in the same fashion. In fact, at first thought, the idea of having neighboring SMCs expressing different myosin isoforms that result in distinctly different shortening velocities and perhaps different amounts of force production appears to be an unreasonable model. But the fact that just such heterogeneity often exists supports the view that this arrangement likely provides a highly beneficial and optimal functional diversity. Variations in SMC lengths and mechanical properties in a given organ could allow a certain fraction of cells to be at their optimal length over the entire functional length range of the organ. One speculative model is that a mosaic distribution of SMCs expressing fast (SMB) and slow (SMA) myosins would allow the faster contracting cells (expressing SMB MHC) to pull on and activate the slower contracting cells (expressing SMA MHC) (35) by stimulating mechanosensitive ion channels (i.e., cause a myogenic contraction) (61, 62). This could increase the rate of activation across the large number of SMCs in organs like the stomach. Alternatively, SMCs expressing fast myosin might be located near the interstitial cells of Cajal to increase the rate of propagation of depolarization to contractile activity between SMCs in organs of the digestive tract (see Ref. 127 for review of interstitial cells of Cajal as pacemaker cells). While these ideas remain speculative because of the current paucity of definitive data, they are all testable hypotheses.

Do SM and NM Myosin Isoforms Have Unique Functions in SM Physiology?

Role of SM1 versus SM2 MHC: COOH terminal isoforms. There has long been evidence that a specific region(s) of the coiled-coil rod is responsible for filament formation (5, 113, 138). Ikebe et al. (66) reported that for both SM and NM MHCs, in vitro and in vivo, it is the COOH-terminal end of the coiled-coil that is required for filament formation (not the nonhelical portion). In addition, Nakasawa et al. (111) more recently reported that filament formation (at least for NMIIB) is highly dependent on the COOH 248 residues for native assembly features. Within this region, there exist a 35 amino acid subregion (Asp<sup>1729</sup> to Thr<sup>1763</sup>) and a 39 amino acid subregion (Ala<sup>1875</sup> to Ala<sup>1913</sup>) with negative and positive charge clusters, respectively, that are both essential for filament formation at both low and high NaCl concentrations. The latter of these two sequences has similarity to the assembly competence domain of skeletal muscle myosin (23, 143). While there are numerous substitutions between the NM and SM myosin rod domains that may thereby alter filament formation between these different isoforms, the rod domains of the SM isoforms are identical with the exception of the COOH-terminal alter-
natively spliced 43 amino acids. Thus, any differences in function between SM1 and SM2 MHC would have to come from this nonhelical COOH-terminal difference.

Myosin filament formation and stability is enhanced by the presence of the longer COOH-terminal nonhelical tail piece in SM and NM MHC (64, 121). This appears counterintuitive because the strict three-dimensional and charge distributions that are known to exist in an α-helix, which allows the α-helical coiled-coil pairing and likely permits the thick filament aggregation to occur, do not exist in these terminal non-coiled regions. Work from our laboratory (98) can also be interpreted as being consistent with SM2 MHC forming less stable filaments. MHC tail isoform expression and cell shortening were examined in freshly isolated single SMCs. We reported a direct correlation between increasing SM2/1 MHC isoform ratio and a shorter final cell length following unloaded contraction. A possible hypothesis for this would be that the SM2 MHC (which has the shorter nonhelical tail piece) forms less stable filaments. Thus, cells expressing a greater amount of this isoform would have less stable filaments that could disassemble upon cell shortening and thus not generate as large an internal resistance or reform in a new location allowing further shortening.

These data suggest that either 1) nonhelical rod domains do not disrupt filament formation or have nothing to do with the extent of cell shortening, 2) the data and or conclusions drawn from the data from one of these studies is not correct, or 3) our
thinking of how thick filaments are organized needs to be revisited. There are data consistent with this idea of labile thick filaments. Smolensky et al. (140) reported that increases in muscle birefringence are correlated with electron microscopy work measuring increases in thick filament densities with stimulation and and/or increased muscle stretching. These changes can occur on a time scale (in s) that is physiologically relevant. There have been similar reports of increases in SM myosin filament content in SM tissues (tracheal and anococcygeus) with activation or decreases with length oscillations by other groups using the same methods (48), quantitative electron microscopy (46, 59, 80, 119, 164), or X-ray diffraction (158) (for reviews, see Refs. 4 and 131). Further work is necessary to test for a causal relationship between thick filament stability/rearrangement and force production or final cell length following shortening. The possible roles the SM1/2 MHC isoforms might play in myosin filament/monomer transitions have also not been examined.

Several papers have suggested that the SM tail isoforms (SM1 and SM2) affect the velocity of shortening in SM tissues. The first of these was by Sparrow et al. (145), which showed a small positive correlation between SM1 expression and unloaded shortening in skinned rat myometrial muscle but a small inverse correlation in intact tissue. Because of the differing results obtained from these two model systems, the authors concluded that there are no obvious differences in the intrinsic properties of these two isoforms. Hewett et al. (60) performed similar experiments in rat uterine tissues from ovariectomized animals and animals treated with or without estrogen. They concluded that a 7% increase in SM1 content with estrogen treatment was significantly correlated with a 230% increase in unloaded shortening velocity and 320% increase in stress. A prediction of Hewett et al.’s data suggests unloaded shortening velocity approaching zero with SM1 expression of less than ~55% and force approaching zero with SM1 MHC expression of less than ~63%. This does not appear to be a valid prediction as there are numerous SM tissues that have SM1 MHC content that is significantly less than 63% (125). It is not clear how to resolve these apparent inconsistencies, but their results also showed a 9% higher MLC20 phosphorylation level in the estrogen-treated animals (that was not statistically significant) that may be part of the answer. They also did not measure MLC17 isoform levels, which others have suggested can affect SM unloaded shortening velocity (see below). In addition, in the same year, Morano et al. (103) also reported on experiments in the rat uterus that included resolving SM and NM MHCs as well as the MLC17 isoforms and concluded that changes in shortening velocity correlated (positively) with MLC17a expression but not with SM MHC expression. More work will be needed to resolve this issue.

Cai et al. (17) reported that the addition into permeabilized SM strips of a 13-amino acid peptide, similar in sequence to

![Image](http://ajpcell.physiology.org/)

**Fig. 7.** Immunofluorescent images of dog stomach antrum (A, longitudinal layer; B, circular layer) and fundus (C, circular layer) tissue sections immunoreacted for the NMB MHC isoform (green) and counterstained for filamentous actin (phalloidin; red) and DNA (DAPI; blue). Colocalization of the red and green channels results in the yellow hue. There is a distinct and dramatic difference in NMB MHC immunoreactivity between individual cells for the expression of NMB MHC. Scale bars = 10 μm.
part of the unique tail region of the SM1 MHC, reduced unloaded shortening velocity. They proposed that this could mimic the effect of the SM1 MHC COOH-terminal interaction via binding to the S2-LMM hinge region of neighboring myosins in the thick filament and altering their function. Other reports looking into possible unique physiological roles of the SM1/2 isoforms have failed to find unique functional properties. Kelly et al. (74) found no correlation between the SM1-to-SM2 ratio and unloaded shortening velocity in freshly isolated single SMCs.

One mechanical feature of the SM that is uniquely distinct from striated muscle is its ability to shorten by 60–80% of its initial length. One possible explanation for this can be found at the filament level. SM myosin filaments have been reported to be side polar without a central “bare zone” rather than bipolar with a bare zone as in striated thick filaments (because they are not helical, they cannot be studied using helical reconstruction) (24–27, 139, 165). SM thick filaments are also reported to have four myosin molecules at each 14.5-nm level as opposed to the three in striated filaments (151). SM and NM MHCs also contain three “skip” residues (rather than four as in skeletal MHC), and the ionic interactions that maximize the intermolecular bonding between the myosin rods are believed to determine a parallel versus antiparallel configuration of thick filaments (64, 96). It is unknown how this variable nonhelical COOH tail piece that does not exist in filaments (146). SM and NM MHCs contain a short and determine a parallel versus antiparallel configuration of thick molecules bonding between the myosin rods are believed to.

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Researchers have used biochemical and molecular methods to express and/or purify SMB and SMA MHC proteins and reported that the myosin-ATPase activity and in vitro actin filament sliding speed for SMB MHC or heavy meromyosin (HMM) is roughly twofold higher than for SMA MHC (28, 75, 123). In 1998, Sweeney et al. (147) expressed chimeric myosin with variable sequences at the region of the SM MHC head isoforms (Fig. 1, loop 1) and found an approximate twofold increase in in vitro motility and actin-ATPase activity that correlated with increasing loop size/flexibility (from 0 to 28 amino acids). These changes were not affected by the MLC17 isoforms present. Lauzon et al. (85, 86) studied expressed SMA (− insert) and SMB (+ insert) SM HMM in a laser trap to show that both isoforms had similar unitary displacements (~10 nm) but the attachment time for SMA was twice that of SMB. At the cellular level, isolated SMCs from the antrum and fundus of the stomach have been used to show a ~10-fold difference in unloaded shortening velocity with extrapolation of the data to cells with 0% and 100% SMB MHC content [actual data from 15–100% SMB (34)]. This range of shortening velocity in cells is roughly five times that reported at the protein level and about half that reported for unloaded shortening in isolated tissue strips (107). The increases in reported values indicative of cell shortening, in studies from the isolated proteins to intact but individual cells and finally to intact tissues, have suggested that the seven-amino acid difference between SMA and SMB can explain only ~1/5 of the difference in unloaded shortening of isolated SMCs and 1/10 of the unloaded shortening of intact SM tissues with SMB expression ranging from ~0% to 100%. This suggests there must be additional factors responsible for the remaining 9/10 of variation in tissue unloaded shortening that is not accounted for by the SMB seven-amino acid insert (34). The list of possibilities for this other 90% of the variability is extensive but certainly would include hypotheses for other myosin subunit isoforms, other protein and protein isoforms, distribution and organization of these proteins, and regulatory pathways. An alternative explanation would include differences in the assumptions that are made in interpreting the data from these very diverse types of studies and in how to harmonize how they fit together.

SMB MHC knockout (−/−) mice have been generated to study the role of SMA/B MHC isoforms (8). Bladder strips from wild-type animals produced significantly more force than heterozygous animals (+/−; 50% less SMB MHC expressed) or knockout animals (−/−; no detected SMB MHC) and had a slower velocity of shortening (by tension regeneration following a slack step). In 2003, this group also reported that the presence of the SMB MHC isoform increases the rate of bronchoconstriction in these animals (153), again suggesting a role for SMB in increasing velocity of cell shortening. In 2004, this same animal model was again used to report that similar changes can be observed in aortic and mesenteric vessels. The investigators found that the −/− animals produce significantly
more force than vessels from wild-type animals. A decrease in aortic SM unloaded shortening was also reported even though the wild-type aorta had little or no SMB MHC, and thus there was no isoform shift between control and \( \text{null} \) aortas (9). In addition, this latter paper (9) also reported increases in calponin, MAPK, and caldesmon expression in the aorta and bladder of \( \text{null} \) animals. In a more recent paper, Patzak et al. (116), working with this same model animal, failed to find any correlation between the presence or absence of SMB MHC and the rate or extent of force generated in perfused afferent and efferent arterioles of \( \text{null} \) wild-type animals. These seemingly disparate results could lead one to conclude that these isoforms are involved in regulating some mechanical properties in some SM tissues and not others or that there are other factors (with this knockout model animal or our models of SM contraction) that need to be resolved. Thus, while exon-specific targeting may be a powerful tool for determining function of specific proteins, these studies have not yet been able to resolve the role of the SMA/B MHC isoforms in SM.

**Role for NM MHC isoforms.** NM MHC has been proposed to be involved in cellular “housekeeping”-type processes, including proliferative, synthetic, and secretory functions, rather than in cell contraction. Cell contraction is usually associated with a differentiated SMC phenotype with high expression of the SM myosin isoforms. In 2000, Morano et al. (102) studied the mechanical and immunological properties of a SM myosin knockout mouse that they generated and reported that NM myosin was responsible for the tonic phase of contraction in the bladder. Bladder strips from knockout animals \( \text{null} \) failed to show the initial strong phasic contraction but did generate a slow tonic contraction that was \( \sim 66\% \) normal bladder tonic force and \( \sim 20\% \) normal bladder peak force. This work was followed up by a subsequent article (89) supporting this finding by showing the presence of 14.3-nm filaments (characteristic of myosin filaments) in these SM MHC knockout animals. They reported no changes in intracellular \( \text{Ca}^{2+} \) transients upon K\(^+\) activation of bladder strips relative to wild type. In these latter experiments, the force produced by \( \text{null} \) mouse bladder strips was \( \sim 11-14\% \) of the intact tissue. While this appears to be an attractive hypothesis to explain these results, it may not be fully justified at this time. The first of these papers reported an \( \sim 34\% \) decrease in tonic force in the \( \text{null} \) mouse bladder, while the second study put this value at \( 86-89\% \). In addition, the tonic forces being reported for the \( \text{null} \) mouse bladder strips are only a fraction of the tonic forces produced in the wild-type mouse bladder \( \text{null} \) and the NM MHC expression decreases to little or nothing in the adult animal (37, 40). Tonic force responses continue to be present in adult wild-type mouse bladder even though the NM MHC expression decreases to little or nothing in the adult animal (37, 40). Tonic force responses have also been reported in other adult rabbit tissues, where there is little or no NM myosin present (36, 47, 54). Finally, neither of these papers adequately address possible compensatory increases in expression of other proteins in these animals that could explain the data equally well. In the adult rabbit bladder, tonic forces are \( 40-50\% \) of the peak force and there is little or no NM MHC present (68, 133). In short, these data, taken together, suggest that NM MHC is not a major factor in tonic contractions of normal adult animal tissues because this isoform is not expressed in detectable levels in these tissues. However, in neonatal tissues, where NM MHC is expressed at very high levels, it may play a role in tonic contractions. In summary, a definitive role for NM MHC in SM force generation is not fully supported, and it appears likely that NM MHC does not play a significant role in contraction of adult SM tissues.

While NM MHC isoforms are very similar to SM MHC isoforms in size and sequence, there are some interesting differences. The NMC isoform is the most dissimilar from the SM MHC isoforms (Fig. 3). NM MHC isoforms all have different loop I sizes/sequences than either of the SM MHC isoforms, being shorter (NMA) or having a Pro(s) substitution (NMB and NMC). All three NM MHC isoforms also have a Pro for Ala substitution in the converter domain and variable nonhelical COOH-terminal lengths (NMA is shorter than SM1 by 5 amino acids and NMB and NMC are longer than SM1 by 4 and 6 amino acids, respectively). The largest difference is in NMC MHC, which has an additional 20 amino acids at the NH\(_2\) terminus (that is not present in any of the other NM or SM MHC isoforms) and a substitution of the next 26 NH\(_2\)-terminal amino acids before the first conserved amino acid is reached. This longer NH\(_2\) terminus in NMC MHC could inhibit the range of motion of the lever arm by its location at the motor domain-lever arm junction. For example, if the extra 20 amino acids remain on the surface of the molecule, they could sterically limit the range of motion of the lever arm by interacting with MLC\(_{17}\) and thus reduce the step size or alter the kinetics of this isoform. The converse scenario has been reported for brush border myosin 1, which does not have the NH\(_2\)-terminal extension present in myosin IIs and has a 50% greater angular swing than does, for example, SM myosin (69). The range of angular swing, the length of the lever arm, and lever arm stiffness are all variables that will affect the step size or unitary displacement due to a single cross-bridge cycle. Testing hypotheses based on altering these variables can determine the viability of the lever arm model, add to our understanding of muscle contraction specifically, and aid in understanding the function of the other classes of myosin (157).

**Role for MLC\(_{17a/b}\) and MLC\(_{20}\) isoforms.** An alternative explanation for what else might be affecting unloaded shortening velocity in SM tissues would be the MLC\(_{17a/b}\) isoforms. In 1988, Helper et al. (58) reported a twofold higher Mg\(^{2+}\)-ATPase activity in myosin purified from the porcine stomach (MLC\(_{17b} = 0\) ) than from the porcine aorta (MLC\(_{17b} = 40\%\) ). This difference could also be observed using a myosin S1 head preparation from these tissues, suggesting that the difference is not due to differences in the MHC tail isoforms (SM1 and SM2), but does not rule out SMA/B differences, which are significant between these organs. In 1991, Malmqvist and Arner (94) showed a reverse relationship between unloaded shortening velocity and relative MLC\(_{17}\) isoform content in SM from various SM tissues and species. In 1992, Hasegawa and Morita (56) made reconstructed aortic myosin and reported a nonlinear 20-fold decrease in actin-activated ATPase activity as MLC\(_{17}\) increased from 23% to 81%. The following year, Morano et al. (103) also reported on experiments in the rat uterus that included resolving SM and NM MHCs as well as MLC\(_{17}\) isoforms and concluded that changes in shortening velocity correlated (positively) with MLC\(_{17a}\) expression but not with SM MHC expression. A prediction of their correlation suggests unloaded shortening approaching zero at a MLC\(_{17a}\) content of less than \( \sim 21\% \), which is a level that is not normally found in SM tissues, but is inconsistent with other studies (see
SMB expression (33,34). These latter reports imply that the and protein levels and then measuring mRNA levels) but a MLC20 isoforms have been reported. It is now well accepted acids (from experimental perturbation or pathological tissues) the variable sizes of section, but it is intriguing because a possible interaction between is also the opening for the nucleotide binding cleft (30). It is reveals that the MLC17 subunits can approach the 25/50-kDa shortening in SM tissues. X-ray crystallography data have approximately twofold higher in vitro motility speed and Mg\(^{2+}\)-ATPase activity in expressed SMB HMM regardless of the MLC17 isoforms present (pure MLC17a or pure MLC17b). In addition, as mentioned above, Sweeney et al. (147) reported that in vitro motility sliding velocity and actin-ATPase activity correlates with increasing loop size/flexibility but were not affected by the MLC17 isoforms present. We also have looked at these correlations in freshly dissociated SMCs and found a lack of correlation of single SMC unloaded shortening velocity and the MLC17a/b isoform ratio (as determined by correlating mRNA and protein levels and then measuring mRNA levels) but a significant correlation of unloaded shortening velocity with SMB expression (33,34). These latter reports imply that the earlier studies suggesting that the MLC17 isoforms present affect shortening velocity were confounded by differences in MHC SMA/B isoform differences in the tissue sources used to isolate the myosin. This would be a simple solution to the controversy except that it does not apply to the Hasegawa and Morita study (56) because the MHC used was all from the same source.

The approximate 10-fold difference between purified protein ATPase activity or in vitro motility/unloaded shortening velocity and unloaded shortening velocity from tissue experiments leaves the possibility (albeit somewhat weak) for the MLC17 isoforms to still have a role in determining unloaded shortening in SM tissues. X-ray crystallography data have revealed that the MLC17 subunits can approach the 25/50-kDa loop 1 (location of the NH2-terminal SMA/B isoforms), which is also the opening for the nucleotide binding cleft (30). It is unclear whether this is a physiologically relevant conformation, but it is intriguing because a possible interaction between the variable sizes of loop 1 (SMA/B isoforms) and MLC17a/b isoforms could help resolve the apparent discrepancy in the data for a possible role of the MLC17 isoforms in the regulation of unloaded shortening velocity in SM tissues. The presence or absence of the essential MLCs or mutations of single amino acids (from experimental perturbation or pathological tissues) have been reported to significantly reduce force generation by these myosins (63, 120, 156).

No known unique functional differences for SM and NM MLC20 isoforms have been reported. It is now well accepted that phosphorylation of MLC20 is the primary means of regulation of SM myosin function (1–3, 11, 13, 14, 20, 76, 77, 141, 142). It remains unclear precisely how MLC20 phosphorylation activates actomyosin-ATPase activity, but there have been a number of fairly recent studies reporting significant conformational changes of the S1 head domain between the unphosphorylated and phosphorylated states (88, 112, 122, 159, 160). In the dephosphorylated state, the actin binding domain of one myosin S1 head is brought in close approximation to (binds?) the converter domain of the second myosin S1 head. Thus, it is believed that the first head cannot bind to actin because its actin binding domain is blocked. The second head cannot function because its converter domain is immobilized by the first head binding to it. This would also prevent the conformational changes required for substrate release from the nucleotide cleft. It remains unclear how the addition/removal of a P, on Ser19 brings these changes about. Sherwood et al. (135) reported that a point mutation in the skeletal regulatory light chain (Leu109 to Phe109) resulted in a twofold reduction in lever step size with no other changes. This suggests that, in addition to whatever else the MLCs may be doing, they are providing flexural rigidity to the lever arm that determines the effective step size of the lever arm. Flexure of the lever arm (and variable flexure due to differential binding of the MLC17 and/or MLC20 isoforms) may allow movement of the lever arm in specific physiological conditions that have not been observed in crystal structures.

Summary and Perspective

SMCs play a vital role in the normal functioning of all tissues and organs either indirectly (for example, the vascular system provides nutrients and hormones to and removes metabolites from all tissues) or directly as the primary tissue type of the organ itself. SMCs exist as bundles of cells in a tissue or organ and have historically been treated as a syncitium of uniform cells. Whether they are electrically coupled (unitary) or not (multunit), they have generally been thought to function in a uniform fashion. While it is clear that SMCs exist as populations of cells in tissues, the degree and effect of cell heterogeneity within different tissues remain less clear. There is an ever-increasing body of data that shows that not all SMCs are the same; that SMCs have unique anatomical, biochemical, and physiological properties; and that heterogeneous distribution of myosin within tissues may be prevalent. Structural, mechanical, and biochemical data collected from myosin molecules (subunits) have significantly increased our understanding of how myosin does what it does and which isoform domains are critical for altered activity. However, differences identified in SMC and tissue/organ contractile properties may result from differences in the experimental protocols and procedures used. Nevertheless, it also appears that many of these differences reflect actual heterogeneities within the different SMCs of these hollow organ systems. And herein lies the conundrum. To understand how individual myosin isoforms or SMCs function, they need to be isolated from one another. However, the process itself of isolating single SMCs, for example, can cause changes to the intercellular connections between the cells. These changes have been shown to alter the cytoskeletal organization of freshly isolated SMCs (39) and may cause other significant intracellular changes. Tissue and organ experiments alone cannot elucidate how protein isoforms or individual cells function in tissues with a heterogeneous population of SMCs, but isolated protein and cell experiments (including cultured cell studies) may have little relevance to in vivo physiology. Experiments on proteins, freshly isolated single cells, and cultured cells are of critical importance for furthering our understanding of individual SMC function, but
without parallel experiments in intact tissues and organs, there is no guarantee that this single protein/cell function has any relevance to whole organ and whole animal physiology. Sound experimental design and proper controls, as always, will allow us to bridge this gap and further our understanding of the functional significance of the myosin isoforms and heterogeneous SM tissues. While the molecular age has provided dramatic advances in our understanding of myosin II structure and function, application of this knowledge to advance our understanding of the physiology of hollow organ contractile function will require integration of work done at the molecular, subcellular, cellular (culture and freshly isolated cells), intact tissue, and intact organ levels.

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REFERENCES

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