Expression of the SM22α Promoter in Transgenic Mice Provides Evidence for Distinct Transcriptional Regulatory Programs in Vascular and Visceral Smooth Muscle Cells

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Abstract. SM22α is a putative calcium-binding protein that is expressed in cardiac, smooth, and skeletal muscle lineages during mouse embryogenesis and in adult smooth muscle cells (SMC). To define the mechanisms that regulate smooth muscle-specific gene transcription, we isolated the SM22α gene and analyzed its 5'-flanking region for elements that direct smooth muscle expression in transgenic mice. Using a series of promoter deletions, a region of the SM22α promoter containing 445 base pairs of 5'-flanking sequence was found to be sufficient to direct the specific expression of a lacZ transgene in mouse embryos in the vascular smooth, cardiac, and skeletal muscle lineages in a temporal pattern similar to that of the endogenous SM22α gene. However, in contrast to the endogenous gene, transgene expression was not detected in venous, nor visceral SMCs. This SM22α-lacZ transgene was therefore able to distinguish between the transcriptional regulatory programs that control gene expression in vascular and visceral SMCs and revealed heretofore unrecognized differences between these SMC types. These results suggest that distinct transcriptional regulatory programs control muscle gene expression in vascular and visceral SMCs.

Unlike skeletal and cardiac muscle cells, which differentiate irreversibly, SMCs exhibit phenotypic plasticity and can readily undergo transitions between the proliferative and differentiated states (Glukhova et al., 1991; Frid et al., 1992; Schwartz et al., 1995). This modulation is apparent in progressively cultured SMCs in which expression of several contractile protein genes is extinguished as the cells acquire a synthetic, proliferative phenotype (Chambley-Campbell, 1981; Owens, 1986; Thyberg, 1987). Because of this plasticity, the properties of SMCs in tissue culture may not reflect those in vivo.

To begin to investigate the molecular mechanisms that regulate SMC gene expression in vivo, we have analyzed the expression of SMC-specific genes during mouse embryogenesis (Miano et al., 1994; Li et al., 1996). We are interested in the regulation of SM22α expression because SM22α is highly expressed in all adult smooth muscle-containing tissues (Lees-Miller et al., 1987; Shanahan et al., 1993; Duband et al., 1993; Duband et al., 1993). Previous studies indicated that SM22α encodes a 22-kD protein (Lees-Miller et al., 1987), which shares sequence homology with Drosophila mp20 (Ayme-Southgate et al., 1989) and NP25 (Ren, 1994), as well as calponin, a troponin T-like protein that interacts with F-actin, tropomyosin, and calmodulin (Duband et al., 1993). Recently, we reported that SM22α is expressed in cardiac, smooth, and skeletal muscle cells during early em-
bryogenesis but becomes restricted to smooth muscle lineages at late embryonic stages and throughout adulthood (Li et al., 1996).

Here we isolated the mouse SM22α gene and analyzed the regulation of the SM22α promoter in transgenic mice as well as in tissue culture cells. Our results demonstrated that the proximal promoter of SM22α was sufficient to direct expression of a reporter gene in cultured SMCs. In transgenic mice, this promoter exhibited a temporospatial expression pattern similar to that of endogenous SM22α transcripts except that transgene expression was not detected in visceral or venous SMCs during embryogenesis. These results reveal previously unrecognized heterogeneity among arterial, venous, and visceral SMCs and suggest that distinct transcriptional regulatory mechanisms exist for the control of SM22α gene expression in different SMC lineages.

Materials and Methods

Isolation and Characterization of Mouse SM22α Genomic Clones

An SM22α genomic clone was isolated by screening an SV129 mouse genomic library (Stratagene Corp., La Jolla, CA) using the 1,078-bp mouse SM22α cDNA as a probe (Li et al., 1996). Southern blot analysis of mouse genomic DNA and the SM22α genomic clone showed the same restriction maps, indicating that the genomic clone was not rearranged. DNA was hybridized on duplicate filter lifts overnight at 42°C in 50% formamide, 5× SSC, 5× Denhardt's solution, 0.05 M Na2HPO4, pH 7.0, 0.1% SDS, 100 μg/ml of salmon sperm DNA. After washing in 1× SSC, 0.1% SDS, positive plaques were carried through three successive rounds of screening. One genomic clone with a 15-kb insert that contained the entire SM22α gene and 5′ flanking sequences was chosen for further characterization.

The intron/exon organization of the SM22α gene was determined by sequence comparison between genomic DNA and cDNA, and by PCR using primers from the cDNA sequence. The intron-exon boundaries were consistent with the AG/ GT splicing consensus sequence.

RNase Protection Analysis

Total RNA from adult mouse tissues and cell lines was isolated by the acid phenol protocol (Chomczynski and Sacchi, 1987). SM22α transcripts were detected by RNase protection using the Maxi-Script and RPA kits (Ambion, Austin, TX). Approximately 15 μg of total RNA was hybridized to an in vitro transcribed SM22α riboprobe (~1 × 10⁶ cpm) corresponding to a 275-bp PCR product described previously (Li et al., 1996).

Generation of SM22α-Luciferase and SM22α-lacZ Reporters

The 15-kb SM22α genomic phage clone was excised by digestion with NotI and subcloned into pBluescript SKII+ (Stratagene Corp.) for further analysis. Initially, a 3,893-bp BglII fragment was subcloned into the pBluescript SKII+ (Nordean, 1988) and pBS-lacZ (Cheng et al., 1992) vectors, yielding the plasmids, pSM2735/1093-luc and pSM2735/1093-lacZ, respectively. This BglII fragment contained 2,735-bp of 5′ flanking region, the 64-bp sequence +41 to +62 with a SalI site engineered at the 3′ end. This PCR fragment was cloned into the TA vector (Invitrogen, San Diego, CA), was located at −2,735; primer B was resolved and complementary to sequence +41 to +62 with a SalI site engineered at the 3′ end. This PCR fragment was cloned into the TA vector (Invitrogen, San Diego, CA), yielding the plasmid pSM2735-TA. The insert was then excised by digestion with BamHI and SalI and the resulting BamHI/SalI fragment, corresponding to sequences from −2,735 to +62, was cloned into pBluescript and pBS-lacZ to yield the plasmids pSM2735-luc and pSM2735-lacZ. A 1,406-bp EcoRI/SalI fragment and a 808-bp SalI/SalI fragment were excised from the pSM2735-TA construct and subcloned into pBluescript and pBS-lacZ, resulting in constructs pSM1343-luc, pSM445-luc, pSM1343-lacZ, and pSM445-lacZ. Finally, a fragment containing 144-bp of 5′-flanking sequence and 62 bp of exon 1 was generated by PCR and subcloned into pBS-lacZ, resulting in the construct pSM144-lacZ. The orientations and identities of the inserted fragments in all constructs were confirmed by sequencing.

Transfection and Luciferase Assays

The primary rat aortic SMC cells (gift from Dr. M. Majesky, Baylor College of Medicine, Houston, TX), 10T1/2 and F9 cells were split and seeded in 6 cm dishes. After 24 h, cells were ~70% confluent before transfection. F9 cells were transfected at ~50% confluence. 5 μg of each luciferase reporter was transfected. Transient transfections were performed by calcium phosphate precipitation as described previously (Li et al., 1992). 48 h later, cells were harvested in 300 μl 0.1 M Tris-HCl (pH 7.8) containing 1 mM DTT. After three cycles of freeze/thaw, cells were spun for 5 min at 4°C. Aliquots (~20 μl) of supernatant containing equal quantities of protein were mixed with 330 μl of reaction buffer (0.1 M Tris-HCl, pH 7.8, 5 mM ATP, 15 mM MgSO4, and 1 mM DTT) and 100 μl 1 mM luciferin (Analytic Luminescence Laboratories, San Diego, CA). Transfection efficiency of different cell lines was determined by comparing the expression of pSV-Luc, which contains the SV-40 enhancer and promoter. All transfections were repeated two to six times.

Transgenic Mice

Plasmids pSM2735/1093-lucZ, pSM2735-lacZ, pSM1343-lacZ and pSM445-lacZ were tested for SM22α promoter activity in transgenic mice. Methods for production and analysis of transgenic mice were described previously (Cheng et al., 1992). The same temporospatial expression pattern was observed in multiple independent transgenic lines. For histological analysis, samples were fixed in 2% paraformaldehyde/0.2% glutaraldehyde in PBS at 4°C for 30 min to 2 h (depending on sample size). After rinsing in PBS and staining with X-gal, samples were dehydrated with ethanol and cleared in xylene, and embedded in paraffin. For larger samples, 0.1% sodium deoxycholate and 0.2% NF-40 were added to PBS for rinsing and staining. Embryos were sectioned on a microtome at a thickness of 5–10 μm, and counterstained with hematoxylin and eosin.

To better visualize the vasculature of the embryo, stained embryos were dehydrated in 100% methanol for 2 d and cleared in a solution of 2 vol of benzyl benzoate per volume of benzyl alcohol for 1–3 h before photography.

Results

Characterization of the SM22α Gene

To begin to study the regulatory mechanisms that control SM22α expression, we isolated the mouse SM22α gene and characterized its structure. As reported previously, the gene spans 5.9 kb and contains 5 exons (Solway et al., 1995). The transcriptional initiation site for SM22α was determined by primer extension and RNase protection and agreed with the results reported by Solway et al. (1995).

The sequence, extending from −2,735 through the first exon to +1,093 bp in the first intron was determined (GenBank EMBL/DDJB accession number U36589). An AT-rich sequence, TTTAAA, which could serve as a TATA box, was located at −28 bp. Sequence analysis revealed a number of sequence motifs in the 5′-flanking region of the gene that have been implicated in transcriptional regulation of smooth, skeletal, and cardiac muscle genes (Solway et al., 1995; and data not shown). In particular, two CArG boxes, CC(A/T)GG, (Gustafson et al., 1988), are present 150- and 274-bp upstream of the transcription initiation site.
Identification of the SM22α 5' Flanking Region That Confers the Transcriptional Activity In Vitro

To choose a cell line suitable for analysis of the SM22α promoter activity, the SM22α expression profile in different cell lines was determined by RNase protection assays. Although SM22α expression was restricted to SMCs in adult mouse tissues (Li et al., 1996), SM22α transcripts were detected in cell lines derived from different origins, including the 10T1/2 and 3T3 fibroblast cell lines, the BC3H1 muscle cell line, which has properties of smooth and skeletal muscle (Schubert et al., 1974; Edmondson and Olson, 1989), the C2 skeletal muscle cell line, and the P19 embryonal carcinoma cell line (Fig. 1). Expression of SM22α transcripts was also detected in primary rat aortic SMCs (not shown). However, no expression was detected in the F9 teratocarcinoma cell line. In the BC3H1, C2, and P19 cell lines, we observed no difference in SM22α expression in differentiated or undifferentiated cells.

As a first step in identifying cis-regulatory elements that may be responsible for the transcriptional regulation of SM22α, we created a series of SM22α-luciferase reporter genes using DNA fragments that extended varying distances upstream from the first exon. These reporters were tested for expression by transient transfection in primary rat aortic SMCs. The reporter pSM2735-1uc, which extends from the first exon to -2,735 bp, was expressed at a level similar to pSM445-1uc, indicating that sequences between the first exon and -445 bp are sufficient to confer transcriptional activity in cultured SMCs (Fig. 2 A). The reporter pSM1343-1uc was expressed at a slightly higher level than pSM2735-1uc, suggesting the possible existence of a negative element between -2,735 and -1,343 bp. Deletion from -445 to -144 resulted in a dramatic decrease in promoter activity, indicating that sequences in this region are important for SM22α transcription.

To determine whether the isolated promoter was active in other cell types, pSM2735-1uc was also tested in 10T1/2 fibroblasts and F9 teratocarcinoma cells (Fig. 2 B). This reporter was expressed at a threefold higher level in SMCs than in 10T1/2 cells and was inactive in F9 cells, in agreement with the expression of SM22α transcripts in these cell types (Fig. 2 B). The construct pSM445-1uc was expressed similarly to pSM2735-1uc in the different cell types (not shown). Both of the above SM22α-luciferase were also active in COS cells (not shown). These results suggest that sequences in the 445-bp proximal promoter of SM22α are sufficient to provide transcriptional activity in tissue culture cells. In a previous study, SM22α was reported to be active only in SMCs in vitro and not in 3T3, COS, or HepG2 (Solway et al., 1995).

Generation of Transgenic Mice Harboring SM22α-lacZ Transgenes

To determine whether the SM22α regulatory sequences tested in tissue culture cells were sufficient to direct appropriate expression in vivo, we generated two lacZ reporter genes: one that extended from -2,735 to +62 bp
(pSM2735-lacZ), the other from -2,735 to 1,093 bp in the first intron (pSM2735/1093-lacZ). These reporter genes were used to generate transgenic mice that were examined for lacZ expression as founders and stable transgenic lines. Both constructs showed comparable expression patterns.

The expression pattern of lacZ in one of the representative transgenic lines harboring pSM2735/1093-lacZ is shown in Fig. 3. Whole mount-staining for lacZ expression revealed that transgene expression was first detected in the primitive heart tube at E8.75 (Fig. 3 A), consistent with the initial expression of SM22α transcripts detected by in situ hybridization (Li et al., 1996). At E9.0, the expression of lacZ in the bulbus cordis and outflow tract increased and expression began to be detected in the dorsal aorta (Fig. 3 B). At E10.0 and 11.5, lacZ expression increased in the dorsal aorta, aortic arches and structures of the heart including the bulbus cordis and the truncus arteriosus (Fig. 3, C and D). Expression in the rostral somites was detectable at E9.5 at a low level (not shown), but became apparent at E10.0 to 12.5 in a rostral-to-caudal fashion (Fig. 3, C and D). However, expression in the somites was transient and only lasted for 2–3 d in each somite. Beginning at E13.5, expression in the heart and somites diminished (Fig. 3 E). LacZ staining marked all major vessels in the head and trunk region at this stage (Fig. 3 E). Transgene expression in the vasculature increased continuously through E14.5 (Fig. 3 F) and E15.5 (not shown). During these stages, expression in intercostal vessels could be seen clearly. At all stages examined, the expression of SM22α-lacZ transgene overlapped with the expression of SM22α transcripts in the vascular smooth, cardiac, and skeletal muscle lineages, with no apparent delay between expression of SM22α transcripts and the transgene.

The primitive heart tube was the earliest site of transgene expression (Fig. 3 A). However, in contrast to the endogenous SM22α gene which was expressed at comparable levels in the bulbus cordis (future right ventricle) and the ventricle of the primitive heart tube (Li et al., 1996),

![Figure 3. Expression of the SM22α promoter in transgenic mice.](image-url)
the transgene was expressed only in the bulbus cordis (Fig.
3, A–D). No lacZ expression was detected in the left ventricle in more than five independent transgenic mice analyzed, suggesting that different cis-regulatory elements are required to control gene expression in different regions of the developing heart.

While expression of the transgene was restricted to the bulbus cordis, we observed sporadic expression in cells of the ventricle and atria (not shown). Whether this represents migrating cells or a heterogenous population of muscle cells in the heart region remains to be determined.

Histological analysis of transverse and frontal sections of E11.5 embryos revealed the expression of the transgene in the myocardium, myotomes, and aorta (Fig. 4, A–C). At E11.5, lacZ was clearly expressed in the trabeculated wall of the bulbus cordis and in the outflow tract (Fig. 4, A and B). However, the bulbar ridges within the outflow tract of the heart, which form the aortico–pulmonary spiral septum, did not show lacZ expression (Fig. 4 B). At this stage, lacZ expression was also detected in the common carotid arteries and dorsal aorta, demarcating the muscle layer of the vessels (Fig. 4, A and C). LacZ expression in the somites was restricted to the myotomal region (Fig. 4 C), where the endogenous gene was expressed (Li et al., 1996).

Specific Expression of the SM22α-lacZ Transgene in Arterial SMCs during Embryogenesis

By E13.5, lacZ activity became restricted to the major vessels throughout the embryo (Fig. 5 A). LacZ expression also demarcated the newly formed pulmonary trunk, which becomes the outlet of the right ventricle. The junction where the ascending aorta and the left ventricle meet was also marked by transgene expression (Fig. 5, B and C). No expression was detected in the bulbar ridges of the outflow tract in the heart or in the trabeculated myocardium of the right ventricle (Fig. 5 B). A high level of transgene expression was observed in the branches of the pulmonary arteries and the descending aorta (Fig. 5, B and C). Under high magnification, transgene expression was seen to specifically demarcate the medial layer of the vessel wall; the transgene was not expressed in endothelial cells which form the single cell layer lining the lumen of the vessel (Fig. 5, D and E). We conclude, therefore, that the SM22α promoter is transcriptionally active in SMCs, but not in endothelial cells of the vessel wall.

In contrast to endogenous SM22α, which is expressed at high levels in visceral SMCs beginning at E13.5, no transgene expression was detected in these cells at this stage of development. Sagittal sections of the abdominal region at 13.5 dpc revealed transgene expression in the umbilical arteries (Fig. 5 A). However, no expression was detected in the hindgut or stomach (Fig. 5 A). At the thoracic level, no expression was observed in the large bronchi or branches of the bronchi in the lung (Fig. 5, B and C; Fig. 6 C).

The absence of transgene expression in visceral SMCs was apparent in transverse sections of embryos at E14.5 (Fig. 6). At this stage, the lumen of the stomach is large, and the structures of the hindgut, bladder, kidney, and liver are easily recognized in the peritoneal cavity (Fig. 6 A). However, lacZ expression was observed only in the descending aorta, umbilical arteries, and femoral arteries, and no expression was detected in the well-defined muscle layers of the stomach, gut or bladder (Fig. 6, A and B). Together, these results show that the regions of the SM22α

Figure 4. Expression of SM22α-lacZ transgene in the bulbus cordis, outflow tract, aorta, and somites of 11.5 d embryos. Transgenic mice harboring pSM2735/1093-lacZ were generated and the resulting embryos were stained for lacZ activity. (A) Frontal section through the ventricle of the heart of an 11.5 d transgenic embryo shows transgene expression in bulbus cordis (bc), outflow tract (ot), and the carotid arteries (ca). (B) Transverse section through the heart region of an 11.5 d embryo revealed lacZ expression in the trabeculated wall of the bulbus cordis (bc), not in the left or right atria (la and ra), ventricle (v), or bulbar ridges (brd) of the heart. (C) Frontal section through the caudal somites of an 11.5 d embryo shows transgene expression in myotome (m) and dorsal aorta (a). Bar, 100 μm.
Figure 5. Expression of SM22a-lacZ transgene in the arterial SMCs of a 13.5 d embryo. Transgenic mice harboring pSM2735/1093-lacZ were generated and the resulting embryos were stained for lacZ activity. (A) A 13.5 d transgenic embryo was stained for LacZ and sagittal sections were stained with hematoxylin and eosin. Transgene expression is seen in major vessels in the head and trunk region. (B) High magnification of A showing the right ventricle of the heart. Transgene expression can be seen in the outflow tract in the heart (ot), branches of the pulmonary arteries (pa), and descending aorta (da). However, no expression was observed in the myocardium of the right ventricle (rv), the bronchus (br), or the vena cava (v). (C) A higher magnification of B shows that the bulbar ridge (brd) in the outflow tract of the heart does not express the transgene. (D and E) High magnification of A in the region of the descending aorta (da) and branches of the pulmonary artery (pa) shows the specific expression of the transgene in the muscle layer (m) around the arteries, not in the endothelial cells (en) lining the lumen. Bars: (A) 500 μm; (B-E) 100 μm.
Figure 6. Absence of expression of the SM22α-lacZ transgene in visceral SMCs of a 14.5 d embryo. Transgenic mice harboring pSM2735/1093-lacZ were generated and the resulting embryos were stained for lacZ activity. A 14.5 d transgenic embryo was stained for lacZ and transverse sections were cut and stained with hematoxylin and eosin. (A) Transverse section at the abdominal level reveals specific expression in abdominal aorta (a) and umbilical arteries (u), and the absence of expression in myometrial layers of the gut (g), stomach (st), and bladder (bl). Specific expression of the transgene in femoral artery (fa) but not the femoral vein (fv) is also observed. (B) Higher magnification of the transverse section at a similar level as A in the 14.5 d embryo. (C) Transverse section at the thoracic level of the same 14.5 d transgenic embryo as in A shows the expression in descending aorta (da) and branches of pulmonary arteries (pa), but not in bronchus (br) or branches of the bronchus in the lung. k, kidney; la, left atrium; lv, liver; ra, right atrium; v, ventricle. Bar, 100 µm.
promoter we tested were active in vascular, but not visceral SMCs, suggesting that SM22α expression in visceral SMCs is controlled by regulatory elements separable from those for vascular SMC expression.

**Specific Expression of the SM22α Promoter in Conductive Arterial, But Not Venous SMCs**

It is well established that SMCs are heterogenous with respect to embryonic origin. Even within the vasculature, SMCs are further specified into conductive arterial, non-conductive, and venous SMCs. When examining transgene expression in the venous system, we observed that the transgene was not expressed in the inferior vena cava at 13.5 dpc (Fig. 5 B). Similarly, no transgene expression was seen in the cardinal vein, nor the femoral vein at E14.5 (Fig. 6). This contrasts with the expression of the endogenous SM22α gene, which is expressed in SMCs in all of these structures. Although the femoral vein is very small in size, SM22α transcripts are clearly detectable (Li et al., 1996). The absence of transgene expression in venous SMCs was apparent at adult stages. Analysis of adult heart from SM22α-lacZ transgenics revealed lacZ expression in the descending aorta, but not in the inferior vena cava or esophagus (Fig. 7 A). This observation was confirmed by histological analysis of transverse sections of the same heart (Fig. 7 B).

In a frontal view of the heart, transgene expression was clearly present in the ascending aorta, aortic arch, left and right common carotid arteries, and right subclavian artery (Fig. 7 C). No expression was detected in the veins penetrating the lung. We also did not detect expression in the coronary arteries passing through the heart. The failure to detect LacZ expression in coronary arteries, veins and visceral organs suggests that distinct regulatory mechanisms direct SM22α gene expression in different smooth muscle lineages.

**The 445 bp of 5′-Flanking Sequence Is Sufficient to Provide Temporospatial Expression Specificity of the SM22α Promoter**

To begin to define the minimal sequences required for expression of SM22α in vivo, we also examined the expression of lacZ reporter constructs containing 1,343 and 445 bp of 5′ flanking sequence. Both constructs showed the same temporospatial expression pattern as the constructs that extended to −2,735 bp (not shown). These results suggest that the expression patterns described above are dependent on proximal promoter sequences and that sequences between −2,735 and −445 bp do not contain essential muscle regulatory elements.

**Discussion**

To begin to define the molecular mechanisms that control smooth muscle gene expression, we examined the regulation of the SM22α promoter in transgenic mice. Our results demonstrate that the 445-bp sequence preceding the transcription initiation site of SM22α is sufficient to direct the expression of a lacZ transgene in cardiac, smooth and skeletal muscle lineages. However, in contrast to the endogenous SM22α gene, which is expressed in all SMC types throughout embryogenesis and adulthood, this promoter was selectively activated in conductive arterial SMCs, but was inactive in venous and visceral SMCs. These results suggest that distinct regulatory mechanisms control SM22α expression in different smooth muscle lineages during embryogenesis.

**Distinct Mechanisms for Regulation of Smooth Muscle Gene Expression in Different Smooth Muscle Lineages**

It is well established that SMCs have at least two distinct embryonic origins, the mesoderm and the neural crest (Schwartz et al., 1990). Embryological studies have demonstrated that SMCs within the dorsal aorta are derived from local mesenchymal cells and that SMC determination occurs before cell condensation around the endothelial channel (Manasek, 1971; Nakamura, 1988), which may signal the differentiation of SMCs. Chick–quail chimera techniques for cell lineage mapping have shown that the regions of the large arteries derived from the branchial arches (systemic aorta, pulmonary arteries, and common carotid arteries) arise from neural crest–ectomesenchymal cells, while the more distal segments of these large arteries may contain a mixture of both ectomesenchymal and mesoderm-derived SMCs (Le Lievre et al., 1975).

Coronary arteries are mesodermal in origin (Le Lievre et al., 1975). However, disruption of the neural crest in birds results in abnormal development of the coronary arteries (Hood and Rosenquist, 1992), suggesting that the neural crest plays a role in normal coronary artery development. The origins of venous SMCs are not well characterized, but these SMCs are likely to be derived from mesodermal mesenchymal cells. In contrast, visceral SMCs, which are distinct from vascular SMCs in contractile properties and pharmacologic responses (Akerlund, 1994; Zingg et al., 1995), arise from mesoderm-derived local mesenchymal cells, whose proper differentiation appears to depend on epithelial cell–mediated signals (Cunha et al., 1989).

The observation that SMCs are derived from distinct embryonic origins raises several interesting questions regarding the mechanisms that control SMC lineage specification and the molecular basis for the heterogeneity and functional diversity of SMCs. As an initial step towards addressing these issues, we have analyzed SMC gene expression in different muscle types. We observed that the onset of expression of SM22α (Li et al., 1996), SM myosin heavy chain (MHC) (Miano et al., 1994) and calponin (Miano and Olson, 1996) in vascular SMCs preceded that in visceral SMCs. Similarly, SM α-actin is expressed significantly earlier in SMCs at the origin of the coronary arteries than in SMCs of the cardiac outflow arteries (Hood and Rosenquist, 1992). In SMCs derived from different physiological or pathological conditions, gene expression was observed to be regulated differentially (Cremona et al., 1995; Schwartz et al., 1995). The expression of calponin, SM22α, and SM α-actin, for example, appears not to be coordinately regulated in human atherosclerotic plaques (Shanahan et al., 1994). These results reveal a previously unrecognized molecular heterogeneity of gene expression in different SMC types and may account for distinct functions of SMCs. The SM22α promoter provides the first molecular marker for dissecting the regulatory mecha-
Figure 7. Expression of the SM22α-lacZ transgene in the descending aorta of an adult transgenic mouse. Transgenic mice harboring pSM2735/1093-lacZ were generated and the resulting embryos were stained for lacZ activity. (A) Gross anatomy of the heart region shown in a dorsal view reveals transgene expression in the descending aorta (da), but not in the vein (v), the esophagus (es) or the lung (lu). (B) Transverse section of the heart in A shows transgene expression in the muscle wall of the descending aorta (da), but not in the vein (v), nor in the esophagus (es). (C) A frontal view of the heart demonstrates transgene expression in ascending aorta, aortic arch, and left common carotid artery (lcc), right common carotid artery (rcc), and the right subclavian artery (rsa). No transgene expression was detected in veins entering the lung or in the coronary arteries (c). Bar, 200 μm.

Li et al. Expression of the SM22α Promoter in Transgenic Mice
gene expression remain largely unknown. Skeletal muscle gene expression is controlled by a family of skeletal muscle-specific basic-helix-loop-helix (bHLH) proteins, MyoD, Myogenin, Myf5, and MRF4, which activate muscle gene expression by binding to the E box consensus sequence (CANNTG) in the control regions of muscle structural genes (Olson, 1990; Weintraub et al., 1991;). There are no E boxes in the 445-bp SM22α promoter that directs cell type–restricted restriction, suggesting that bHLH factors are not essential for SM22α gene expression.

The SM22α Promoter Reveals Different Expression Specificity In Vitro and In Vivo

Whereas endogenous SM22α is expressed specifically in myogenic lineages in vivo, we detected SM22α transcripts in a variety of muscle and nonmuscle cell types in culture. The SM22α promoter was also less tissue restricted in its expression in cultured cells than might be expected from the strict cell-type specificity of SM22α expression in vivo. Deletion analysis indicated that the 445-bp proximal promoter of SM22α was sufficient to direct transcription in these different tissue culture cell types. This finding contrasts with a recent study in which the proximal SM22α promoter also offers an opportunity to genetically manipulate the physiology and pathology of the cardiovascular system by directing genes involved in cell growth and differentiation into the cardiovascular system.

We thank P. Overbeek and G. Schuster for assistance in generating transgenes, which exclusively mark the bulbus cordis in SM22α-expressing cell types in which the transgene expression is controlled by a family of skeletal muscle-specific basic-helix-loop-helix (bHLH) proteins, MyoD, Myogenin, Myf5, and MRF4, which activate muscle gene expression by binding to the E box consensus sequence (CANNTG) in the control regions of muscle structural genes (Olson, 1990; Weintraub et al., 1991;). There are no E boxes in the 445-bp SM22α promoter that directs cell type–restricted restriction, suggesting that bHLH factors are not essential for SM22α gene expression.

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Studies of SMC gene promoters in tissue culture cells have provided useful information about the cis-acting elements that control SMC gene expression in vitro (Kahari et al., 1990; Katoh et al., 1994; Shimizu et al., 1995). However, whether these regulatory elements are also sufficient to confer temporospatial expression specificity in vivo, where higher orders of transcription control exist (Felsenfeld, 1992), remains unknown because there have been no previous studies demonstrating SMC-specific gene expression in transgenic mice.

Transcriptional Control of Muscle Gene Expression

Significant progress has been made toward identifying the transcription factors that control skeletal and cardiac muscle gene expression (Olson, 1993; Buckingham, 1994). However, the mechanisms that regulate SMC-specific gene expression remain largely unknown. Skeletal muscle

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