Mechanisms of Transcriptional Regulation of Gene Expression in Smooth Muscle Cells

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Smooth muscle (SM) plays an active role in a multitude of physiological and pathological vascular processes ranging from vasculogenesis to arteriosclerosis. Development of therapeutic strategies targeted specifically against an SM process requires an understanding of the molecular basis of the involved molecules and reactions. Investigation of regulatory mechanisms underlying gene expression is of vital importance, given its pivotal role in regulating these processes. Owing to recent efforts, our understanding of the molecules and reactions involved in gene expression of SM has shown great progress. Still, compared with its distantly related skeletal muscle, which has been thoroughly dissected by both biochemical and genetic studies, little is understood of the SM regulatory program, likely due to a lack of markers of SM that could be used for identifying key determinants of the SM lineage and of an in vitro model of SM differentiation for studies of gene expression. These studies have allowed the development of putative models of molecular mechanisms regulating SM gene expression. This Mini Review will focus on recent advancements in SM biology, with an emphasis on these issues.

SM-Specific Genes: Characterization and Regulation of Gene Expression

Studies on regulatory mechanisms have been focused mainly at the transcription level because it is responsible for dynamic regulation (eg, qualitative and quantitative) of the gene product and also with expectations of analogy to the related skeletal muscle program, which has been shown to be determined mainly at the transcription level by a family of myogenic regulatory factors (eg, MyoD). These factors belong to the basic helix-loop-helix family of DNA-binding transcription factors that bind the CANNTG, known as the E box.1 To date, no known single factor nor group of factors has been isolated that can determine the cellular fate of SM. However, recent studies that have focused on understanding the regulatory mechanisms of SM-specific and -selective expression of genes have pointed to a common DNA-binding element, the CArG box,2 a 10-bp region [CC(A/T)6GG] that has been shown to bind the ubiquitous serum response factor (SRF) and to be a key element involved in SM transcription, suggesting possible unity in underlying mechanisms. Our present knowledge of the mechanisms underlying gene regulation of various SM genes will be described in the following sections.

Structural Proteins

The major structural proteins in SM are the actins and myosins. SM myosin heavy chain shows SM-specific expression at both the RNA and protein levels. The gene encodes two distinct isoforms, SM1 and SM2, by alternative splicing, which are developmentally and pathogenically regulated. SM1 expression is seen in all SM phenotypes, whereas SM2 expression is limited to differentiated SM. SM1 and SM2 are the most stringent markers of the SM lineage. The regulatory mechanisms underlying expression of the SM1/2 gene have been extensively pursued as a model of SM-specific gene expression.

Deletion mutagenesis of rabbit SM1/2 has shown a 107-bp region located −1332 to −1225 bp from the transcription initiation site to be an enhancer region.3 This region is highly conserved across species (eg, the rat and mouse), and known cis-elements or trans-factor binding sites contained in this region include multiple CArG boxes, a GC-rich site, and a nuclear factor-1 binding site.4 The GC box is an element initially shown in the SV40 early promoter to bind the ubiquitous zinc finger transcription factor Sp1 and has been implicated in negative regulation of the SM1/2 gene.5 Studies by other groups on the rabbit promoter have shown an A/T-rich site located −1594 to −1462 bp from the transcription initiation site to bind a distinct member of the myocyte enhancer factor 2 (MEF2) family, a subgroup of transcription factors containing the MADS domain, a DNA-binding domain found common to MCM1, aagam, deficiens, and SRF.6 Studies with the murine SM1/2 gene have shown a tandem CACC box analog in the core promoter of the gene at −61 and −89 bp from the transcription initiation site to be important for transcriptional activation of the gene in SM but not in other cell types.7 The CACC box has been implicated as a cis-element vital for gene expression in other muscle cell types and is conserved among species, also being found in the rat core promoter. Taken collectively, a combined mechanism likely exists, with transcription factors binding the CACC
box, the distal upstream element(s) of the enhancer (eg, CArG and Sp1), and the MEF2 site through direct (eg, protein-protein interactions) or indirect (eg, mediating cofactors) pathways to activate transcription of the gene. Further dissection and confirmation using both biochemical and genetic approaches should verify this association.

Six actin isoforms are known in mammals and are categorized into α, β, and γ types according to their isoelectric properties. SM α-actin is predominantly expressed in vascular SM, and intestinal SM mainly expresses the γ-type isoform. SM α-actin is the first known marker that is developmentally expressed in differentiated SM.

Promoter analysis of the rat SM α-actin gene has shown two CArG elements located within the region 125 bp upstream from the transcription initiation site to be required for cell-specific expression in cultured, vascular SM. Studies of the chicken SM α-actin gene have shown these two CArG boxes to be conserved in the core promoter and for this region to be important for transcriptional activation, although a distinct pattern of regulation seems to exist for SM versus non–SM type cells. The CArG elements are conserved in the core promoters of SM α-actin genes of all investigated species and are also found in the promoters of skeletal and cardiac α-actin genes as well as other muscle-associated genes, further supportive evidence of an important regulatory role for this DNA element.

Regulatory Proteins

Studies on transcriptional regulation of regulatory proteins have been centered on the actin-binding proteins, which include caldesmon, calponin, and SM22α. Caldesmon consists of high- and low-molecular-weight isoforms, which are expressed in differentiated and dedifferentiated SM, respectively. The isoforms, which are thought to be alternative splicing products, show similar properties and contribute to the actomyosin system. On the basis of its unique developmental expression pattern, caldesmon can be used as a phenotypic marker of SM. The promoter of the avian gene has been analyzed, showing a CArG box at −315 bp from the transcription initiation site to be important for transcriptional activity.

Calponin, a 34-kDa protein related to troponin T, is expressed selectively in adult SM. One group has shown that interaction of a cis-acting domain with a methylated DNA-binding transcription repressor may play a regulatory role in the activity of the gene.

SM22α is a 22-kDa protein related to calponin that is expressed in differentiated SM. Along with SM myosin heavy chain, SM22α is the most stringent marker of differentiated SM. Although its function is unknown, absence of additional regulation (eg, alternative splicing) renders SM22α a favorable model for investigation of transcriptional regulation in SM. Numerous groups have analyzed the promoter region. The region −445 bp from the transcription initiation site has been shown to be sufficient for transcriptional activation. Transgenic studies have shown this region to be sufficient to confer arterial SM-specific expression, suggesting that differential regulatory processes in vascular SM may exist. Two CArG boxes located within this region are essential for full promoter activity in cultured SM, but only the single proximal CArG box is necessary for specific expression in developmental stages, which may suggest differential use of transcriptional complexes.

Transcription Factors

Studies focused on isolating transcription factors involved in SM processes have also been pursued during the last several years. The homeobox transcription factors regulate temporal and spatial gene expression during development. Given this property, the isolation of tissue- and lineage-specific homeobox factors has been pursued, with recent identification of homeobox factors expressed in SM. MHomeoX is a mesodermally restricted homeodomain protein that was initially cloned as a factor that bound to the A/T-rich site of the muscle creatine kinase gene enhancer. Hox 1.11 is another homeobox gene whose transcripts are restricted to aortic SM and lung but are undetected in cardiac or skeletal muscle or visceral SM. The GAX gene is also a recently isolated diverged homeobox gene expressed in SM that shows differential cell-cycle expression with downregulation during the G0/G1 transition in vascular SM. Another report has demonstrated the differential expression pattern of homeobox genes during SM differentiation. Whereas homeobox gene expression is absent in adult SM, numerous homeobox genes are expressed selectively in fetal SM (eg, Hox B7, C9, A5, etc). Collectively, these data suggest a possible role for homeobox genes in SM.

Another class of transcription factor being pursued in SM is the zinc finger–type transcription factors. SmILM (Lin-11, Isl-1, Mec-3) is a recently isolated LIM domain transcription factor expressed selectively in aortic SM. The LIM domain is a subclass of zinc finger transcription factors with functional family factors in skeletal and cardiac muscle. GATA-5 and -6 are also a subset of zinc finger transcription factors found in SM. The GATA family of zinc finger transcription factors was first isolated in the hematopoietic system (GATA-1), with future studies showing a family of factors in...
other tissues, including the heart (GATA-4). For these zinc finger transcription factors, further biochemical and genetic analyses should provide insight into their function.

**In Vitro System of SM Cell Differentiation**

A limiting factor of our understanding of SM differentiation has been the lack of a system employable for the investigation of gene regulation in SM. In contrast to the well-understood skeletal muscle, in which the differentiation process from myoblasts to myotubes can be observed and investigated in vitro (eg, C2 cells), a similar system has not been available for SM. Recent efforts have addressed this issue.

Two groups have focused efforts on the murine P19 teratocarcinoma cell, which can be induced to differentiate into different cell types.\(^\text{21}\),\(^\text{22}\) The optimum chemical regimen to induce differentiation of P19 cells into SM cells has been shown to be a high concentration of retinoic acid (1 \(\mu\)mol/L), which also causes concomitant differentiation of these cells into neuronal cells. The SM cells reach differentiated stages (eg, the presence of SM myosin heavy chain SM2 isoform and response to contractile agonists). To overcome the markedly heterogeneous cell population, one group cloned the P19-induced SM cells,\(^\text{21}\) and the other group used a construct to reduce neuronal cell expression by a stable transformant against the POU (P\(_{ou}\)) construct to reduce neuronal cell expression by a stable transformant against the POU (P\(_{ou}\)) construct to reduce neuronal cell expression by a stable transformant against the POU (P\(_{ou}\)) construct to reduce neuronal cell expression by a stable transformant against the POU (P\(_{ou}\)) construct to reduce neuronal cell expression by a stable transformant against the POU (P\(_{ou}\)) construct to reduce neuronal cell expression by a stable transformant against the POU (P\(_{ou}\)) construct to reduce neuronal cell expression by a stable transformant against the POU (P\(_{ou}\)) construct to reduce neuronal cell expression by a stable transformant against the POU (P\(_{ou}\)) construct to reduce neuronal cell expression by a stable 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Although only limited success has been obtained, these systems may be of use as a model for SM differentiation and investigation of developmental regulatory mechanisms. Another group has shown differentiation of neural crest progenitor cells into SM cells by transforming growth factor-\(\beta\) induction. However, because SM \(\alpha\)-actin and calponin, which are less stringent markers of SM, were used as their criteria of SM expression, confirmation of their results with more stringent SM markers (eg, SM myosin heavy chain) may be necessary.\(^\text{23}\)

**Conclusions**

Transcription involves a milieu of DNA-protein/protein-protein interactions that assemble multiprotein complexes at enhancer/core promoter elements, which act in concert to activate the basal RNA polymerase machinery. Since Jacob and Monod\(^\text{24}\) proposed their pioneering operon model more than 30 years ago by describing the bacterial \(lac\) operon to be genetically regulated by upstream regulatory factors, little has changed in our basic concepts of the regulation of transcription processes through DNA-binding factors. Because interactions between transcription factors and promoters at the DNA level are a decisive step in regulating transcription, understanding DNA-protein interactions is an important initial step in understanding regulation of gene expression by transcriptional processes. Studies on SM transcription have been centered on interactions between DNA \(cis\)-elements and their binding factors, given this reasoning. Through studies on a multitude of genes expressed in SM, it seems that the CArG-box is a commonly involved element.

The DNA-protein interaction is just one of the few known reactions necessary for transcription. Indeed, the simplest model of transcription would involve a single DNA element and a sequence-specific binding transcription factor unique to this site. Tissue-specific or development-specific regulation of the single binding reaction between the element and binding factor would be sufficient. However, actual mechanisms are not likely this simple. It is envisioned that a multitude of DNA-protein and protein-protein reactions act in concert, forming functional, regulatory, multiprotein complexes to activate gene transcription. The individual reactions or a combination of such likely confers a unique functional aspect to the transcriptional reaction as a whole. One component may control tissue specificity and another, developmental specificity, while yet another may direct an inducible response (Figure 1). Interactions with the basal RNA polymerase machinery either directly or indirectly (eg, coactivators) will also be necessary to activate transcription. This multicomponent reaction would result in a final output that would dictate whether to switch transcription on or off.

Returning to the role of the CArG box in SM transcription, one can envision the occurrence of the following events centered on this element. Evidence suggests that the ubiquitous SRF binds to the CArG box of SM genes. Given that murine SRF transcripts are expressed in abundance in adult cardiac and skeletal muscle and are enriched in muscle-lineage tissues including SM in early development,\(^\text{25}\) the temporospatial expression of this DNA-binding protein is likely to be an important determinant of lineage-restricted expression. To further regulate tissue- or cell-
specific-gene expression, binding of regulatory proteins by protein-protein interactions to the SRF seems likely to occur. One example of such regulation is seen in the immunoglobulin gene promoter. Interaction of the DNA-binding transcription factor Oct-1 or Oct-2 with the cell-specific coactivator OBF-1/Bob-1 by protein-protein interactions is necessary for lymphoid-restricted immunoglobulin gene expression.\textsuperscript{26}

The discussion so far has been limited to naked DNA, in which DNA is freely accessible to binding by transcription factors. However, in the eukaryotic organism, DNA is closely packed into a nucleosomal array that consists of DNA wrapped around a histone octamer and additional linkage between these units (Figure 2). For transcription to occur in the organism, this chromatin structure must be remodeled. Recent investigations have addressed this field. One event under investigation is that of histone acetylation, in which acetylation of the histone tail leads to remodeling of the tightly packed nucleosomal DNA, thus allowing access and interaction of transcription factors and subsequent activation of transcription. A number of coactivators (eg, p300/CBP [CREB-binding protein]), which transmit signals from sequence-specific activators to the basal transcriptional machinery, have been shown to possess histone acetyltransferase activity. Recent studies have shown that the formation of a multiprotein complex of MyoD with histone acetyltransferases p300 and PCAF (p300/CREB-binding protein-associated factor) is crucial for myogenic differentiation and that EIA inhibition of myogenic differentiation is caused by interference of the interaction between p300 and PCAF.\textsuperscript{27} Conversely, histone deacetylases repress transcription. Thus, unraveling the nucleosomal DNA is also a critical and important aspect in the initiation of the transcription response. The next questions to be answered will be how these chromatin remodeling factors or complexes are regulated.

In closing, we have but a limited understanding of the mechanisms underlying SM processes and have much more to learn (eg, about molecules and reactions). Our investigations of SM transcription (and many other systems as well) have begun by understanding the individual DNA-protein interactions. However simple this approach may seem, this is only one among the many involved reactions. Further protein-protein interactions forming multiprotein transcriptional complexes and remodeling of higher-ordered DNA structure (eg, chromatin) are necessary in addition to other hitherto-unknown reactions. As we unravel how transcription is controlled in SM, one should keep in mind that transcription is the cumulative effect of multiple reactions and components. Elucidation of the critical reactions will allow understanding of the fundamental reactions of this cascade and for model development of a putative backbone for further investigations.

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**References**


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