Multiple Sp1 Binding Sites in the Cardiac/Slow Twitch Muscle Sarcoplasmic Reticulum Ca\(^{2+}\)-ATPase Gene Promoter Are Required for Expression in Sol8 Muscle Cells*

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The rabbit cardiac/slow twitch muscle sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA2) gene encodes a Ca\(^{2+}\) transport pump whose regulation is expressed during skeletal and cardiac muscle development and in response to various pathophysiological and hormonal states. Employing transient transfection analyses in Sol8 muscle cells, we have identified two positive regulatory regions, one distal (−1810 base pair (bp) to −1110 bp) and one proximal (−284 bp to −72 bp), within the SERCA2 promoter. The proximal promoter region from −284 bp to −80 bp was shown to confer muscle-specific expression to a heterologous promoter in Sol8 cells. This region is highly GC-rich containing the consensus sequence for four Sp1 elements (GGGCCG) and three Sp1-like elements (GGAGGG). DNase I footprint analysis with Sol8 nuclear extracts and purified Sp1 protein showed the protection of the seven Sp1 binding sites. In addition, site-directed mutagenesis of the Sp1 consensus sites demonstrated that Sp1 sites are essential for the muscle-specific expression of the SERCA2 promoter. Furthermore, we demonstrate that cotransfection of an Sp1 expression vector together with SERCA2-CAT constructs can up-regulate SERCA2 promoter activity. These results imply that the Sp1 transcription factor plays an important role in the transcriptional regulation of SERCA2 within muscle cells.

SERCA2 encodes two alternatively spliced Ca\(^{2+}\)-ATPase isoforms, SERCA2a (6, 7) and SERCA2b (8, 9), which diverge in the COOH-terminal region (SERCA2a = 4 amino acids; SERCA2b = 49 amino acids). These isoforms differ in Ca\(^{2+}\) affinity, Ca\(^{2+}\) turnover rate, and ATP hydrolysis (10). Functional differences between the two isoforms have been attributed to 12 amino acids located within the carboxyl-terminal region of SERCA2b (11).

SERCA2a, the cardiac/slow twitch Ca\(^{2+}\)-ATPase, is the primary isoform expressed in cardiac muscle, both in the atrium and the ventricle (12–14). The earliest expression of SERCA2a mRNA can be traced to the heart tube of 10-day post-coitum rat embryos (15, 16). During heart development the SERCA2a mRNA level increases gradually from fetal to adult stages (15, 17).

SERCA2a mRNA is also expressed in developing skeletal muscle cells in fetal and neonatal stages (17, 18). However, in fast twitch skeletal muscle the SERCA2a isoform is replaced by the SERCA1 isoform in the adult stage (17). In contrast, the SERCA2a isoform predominates in both fetal and adult slow twitch skeletal muscle tissue. SERCA2b, on the other hand, is found primarily in smooth muscle and non-muscle cells (9, 14, 19, 20).

Our previous studies with the rabbit SERCA2 promoter showed that the upstream region extending to −1110 bp from the transcriptional start site is capable of promoting reporter activity in differentiating C2C12 muscle cells (21). In particular, these studies demonstrated that the proximal promoter region (−284 bp to +350 bp) is highly active in skeletal muscle cells.

The goal of this study is to perform a detailed analysis of SERCA2a proximal promoter elements important for high level expression in muscle cells. In this study, we use a slow twitch muscle cell line, Sol8 (22) (derived from mouse soleus), which expresses high levels of SERCA2a (23) for promoter analyses. Our DNA transfection analyses reveal two positive regulatory regions, one proximal (−284 bp to −72 bp) and one distal (−1810 bp to −1110 bp). We demonstrate that the proximal promoter region (−284 bp to −80 bp) functions like an enhancer element in muscle cells when transferred to a heterologous promoter-reporter system (TK-CAT). This region is highly GC-rich and contains seven Sp1 binding sites. Using DNase I footprint analyses and site-directed mutagenesis of the Sp1 sites, we demonstrate that the Sp1 elements are critical for SERCA2a promoter activity in muscle cells. Furthermore, we show that overexpression of Sp1 can up-regulate SERCA2a promoter activity in Sol8 muscle cells.

EXPERIMENTAL PROCEDURES

Cell Culture—Sol8 (22), a mouse soleus muscle cell line was maintained in Dulbecco’s modified Eagle medium (Life Technologies, Inc.) supplemented with 20% fetal bovine serum (Life Technologies, Inc.). Sol8 myoblasts were induced to differentiate by switching to a medium...
containing 5% horse serum (Life Technologies, Inc.). NIH 3T3, a mouse fibroblast cell line, (obtained from ATCC CCL92) was maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum.

Plasmid Construction—Unidirectional deletions of the SERCA2 promoter were produced using the Erase-a-base system (Promega) in the pBLCAT3 vector (21). The largest promoter construct, p1810-CAT, was produced through ligation of a 700-bp fragment (SalI to EcoRI) of the SERCA2 promoter (from −1810 bp to −1110 bp) to the p1110-CAT construct. Heterologous SERCA2/TK promoter constructs were created by ligating the −284 bp to −80 bp region (blunt HindIII to EcoRI) to pBLCAT2 (24) both 5' (sense and antisense orientation) and 3' (antisense orientation) of the CAT gene. Orientation of the SERCA2 fragment was confirmed by DNA sequencing. The Sp1 expression vector pPACSP1 containing the 2.1-kilobase pair human Sp1 cDNA was kindly provided by Robert Tjian (25). Constructs pBLCAT2, pBLCAT3,
Site-directed Mutagenesis—Mutations to the Sp1 consensus elements were generated using the Altered Sites II in vitro mutagenesis kit (Promega). Mutagenic oligonucleotides containing two nucleotide substitutions were synthesized as follows: SPI, 5'-TCCGGGTTCCTGGGTTCGGTGCGCGGGAGG-3'; SPIII, 5'-GGTGCGCGGGAGGGTTCCGGGGCCTGCGCGG-3'; SPVI, 5'-CCGGGGGGAGGGTTCCGGGGCGCGCCGCCC-3'; and SPVII, 5'-GGGGCCGCGCCGAACGCGCCGCGCTGG-3'.

Mutagenic oligonucleotides were annealed to denatured double-stranded pALTER-284 template along with appropriate ampicillin and tetracycline knockout and recovery oligonucleotides. Synthesis of a mutant strand was performed with T4 DNA polymerase and T4 DNA ligase. Competent ES1301 mutS (restriction 1) cells were co-transformed with the extended product along with R408 helper phage DNA, which allows the production of phagemid DNA from the pALTER vector. JM109 cells were infected with phagemid containing the mutant DNA and selected with the appropriate antibiotic. Isolates were monitored for appropriate antibiotic resistance, and mutations were confirmed by DNA sequencing before cloning into the pBLCAT3 vector. Serial mutations of the Sp1 sites were made progressively following the same protocol.

DNA Transfections and CAT Assays—SERCA2 promoter constructs (20 μg) containing the CAT reporter gene were cotransfected with pMSVβgal (5 μg) into Sol8 and NIH 3T3 cells by the calcium phosphate coprecipitation method (27). To determine the role of Sp1, cotransfections were performed using 5 μg of the Sp1 expression plasmid, pPACSp1 (24). For DNA transfections, Sol8 cells were seeded at 5 x 10^5 cells/10-cm dish, and the calcium phosphate-DNA precipitates were added 24 h later. Myoblasts were incubated with DNA for 5 h, followed by a glycerol shock step, and the replacement of medium. Sol8 myogenesis was induced 12–16 h later with the replacement of 5% horse serum medium. Cells were harvested 48–72 h after transfection, washed twice with phosphate-buffered saline, resuspended in 100 μl of 250 mM Tris, pH 7.5, and lysed through three freeze-thaw cycles. β-Galactosidase activity was determined for each sample (28). CAT activity was assayed according to established procedures and normalized for transfection efficiency as determined by β-galactosidase expression. Data represent the average of three or more independent transfection experiments run in duplicate. CAT activity is represented as the relative CAT activity as compared to promoterless control pBLCAT3 construct activity, unless otherwise indicated.

Gel Mobility Shift Assay—Nuclear extract from Sol8 myoblasts and myotubes were prepared according to Gossett et al. (29). Gel mobility shift assays were performed by incubating labeled DNA with nuclear extract (4 μg) for 30 min at room temperature in 10 μl of Sp1 binding buffer (30) containing 12.5 mM Hepes-KOH, pH 7.5, 6.25 mM MgCl₂, 10% (v/v) glycerol, 0.05% (v/v) Nonidet P-40, 5 μM ZnSO₄, 50 mM KCl, 50 μg/ml bovine serum albumin, and 2 μg of poly(dI)poly(dC). The binding reactions were immediately loaded on to 4% native polyacrylamide gel containing 0.5 x TBE. Electrophoresis was carried out at 4 °C for 3 h at 100 V. The gel was subsequently dried and autoradiographed.

Fig. 2. The SERCA2 proximal promoter (−284 bp to −80 bp) up-regulates the TK-CAT reporter in Sol8 muscle cells. A, schema showing the −284 bp to −80 bp region linked in both 5' (sense and antisense) and 3' (antisense) to the TK-CAT reporter. The locations of Ap2 sites, Sp1 sites, CCAAT box, and GATAA box are illustrated. B, the SERCA2/TK-CAT activity was determined in Sol8 myotubes and NIH 3T3 fibroblasts. The CAT data represent the averages of at least three transient transfection experiments in Sol8 myotubes. The CAT data are represented as the -fold activity over the pBLCAT2 construct activity.

Fig. 3. Nucleotide sequence of the SERCA2 proximal promoter (−284 bp to −72 bp). The consensus binding sequences for the Sp1 transcription factor (sites I, III, VI, and VII) are indicated. Sp1-like (GGGAGG) sequences (sites II, IV, and V) are denoted by an asterisk, and Ap2 consensus sites are also boxed. A 17-bp region from −284 bp to −267 bp responsible for high level expression of the proximal promoter in Sol8 cells is also indicated.
DNase I Footprinting—DNase I footprinting was performed on the 2284 bp to 280 bp promoter region using purified Sp1 protein (Promega) and Sol8 nuclear extracts. Both sense and antisense strand in the presence of Sol8 myocyte nuclear extracts. Lanes 2 and 3 and lanes 6 and 7 correspond to increasing concentrations of nuclear extract in the presence of DNAse I. Lanes 4 and 5 were incubated with DNAse I (D) alone. A Maxam and Gilbert A+G DNA sequence ladder of the same region is shown in lanes 1 and 8. Black boxes indicate protected regions corresponding to Sp1 consensus or Sp1-like sites. The hatched box represents an additional protected region designated CATP1. Lanes are designated 1–8, starting from left to right.

**RESULTS**

SERCA2 Promoter Contains a Proximal and a Distal Positive Regulatory Region Responsible for High Level Expression in Sol8 Muscle Cells—To define critical promoter elements in the SERCA2 gene, a series of SERCA2 promoter deletion constructs linked to the CAT reporter gene (Fig. 1A) were transiently transfected into Sol8 muscle cells, and the reporter solution (200 mM NaCl, 30 mM EDTA, and 1% SDS), phenolized, and ethanol-precipitated. The samples were heat-denatured and loaded on to a 6% sequencing gel. The A+G ladders were generated by the Maxam and Gilbert chemical sequencing method (31).

**Fig. 4.** DNase I footprint analysis of the 2284 bp to 280 bp SERCA2 promoter region with Sol8 myocyte nuclear extract. The 2284 bp to 280 bp DNA fragment of the SERCA2 proximal promoter was used to perform DNase I footprint analysis on both the sense and antisense strand in the presence of Sol8 myocyte nuclear extracts. Lanes 2 and 3 and lanes 6 and 7 correspond to increasing concentrations of nuclear extract in the presence of DNase I. Lanes 4 and 5 were incubated with DNase I (D) alone. A Maxam and Gilbert A+G DNA sequence ladder of the same region is shown in lanes 1 and 8. Black boxes indicate protected regions corresponding to Sp1 consensus or Sp1-like sites. The hatched box represents an additional protected region designated CATP1. Lanes are designated 1–8, starting from left to right.

**Fig. 5.** DNase I footprint analysis of the 2284 bp to 280 bp SERCA2 promoter region using purified Sp1. Labeled probe was incubated with increasing concentrations of purified human Sp1 protein (lanes 3–5 and 9–11) or in the absence of Sp1 (lanes 2 and 8). A Maxam and Gilbert A+G DNA sequence ladder of the same region is shown in lanes 1 and 6 and lanes 7 and 12. Black boxes indicate protected regions that correspond to Sp1 consensus (GGGCGG) sequences or to Sp1-like (GGGAGG) sequences. Lanes are designated 1–12, starting from left to right.
activity was determined (Fig. 1, B and C). Transient transfection analyses demonstrate that DNA sequence extending to −284 bp from the transcriptional start site was able to promote high levels of CAT activity in differentiating Sol8 muscle cells. Interestingly, a short deletion (17 bp) created between −284 bp and −267 bp produced a significant decrease (69%) in activity (Fig. 1 C), suggesting a positive regulatory element is present in this region. Inclusion of sequences within the −490 bp to −562 bp region produced a decrease in promoter activity, indicating that negative regulatory elements are located within this region. However, when upstream DNA sequences between −1810 bp and −1110 bp were included, maximal promoter activity was restored, suggesting the presence of additional positive regulatory elements in this region. From these data, it is evident that the SERCA2 promoter contains at least two positive regulatory regions: a proximal region (−284 bp to −72 bp) and a distal region (−1810 bp to −1110 bp) (Fig. 1 C).

To determine whether SERCA2 promoter activity is restricted to differentiating myotubes, the promoter constructs were transfected into Sol8 myoblasts (undifferentiated) and a fibroblast cell line (NIH3T3), and CAT activity was determined (Fig. 1C). The SERCA2 promoter activity was significantly lower in myoblasts and is negligible in NIH 3T3 fibroblasts (Fig. 1C). Thus, the −284 bp to −80 bp region of the SERCA2 promoter appears to function as a positive enhancer-like regulatory element in muscle cells.

DNase I Footprinting Reveals Protection of Multiple Sp1 Binding Sites within the SERCA2 Proximal (−284 bp to −80 bp) Promoter Region—The proximal promoter region (−284 bp to −80 bp) includes four consensus Sp1 sites (GGGCGG), three Sp1-like sites (GGGAGG), and three putative Ap2 binding sites (Fig. 3). To determine the precise nature of protein binding in the −284 bp to −80 bp DNA fragment, DNase I footprint analysis was carried out using nuclear extracts from Sol8 muscle cells. DNase I footprinting of the sense strand showed strong protection of Sp1 binding sites II–VI, and an additional region designated as CATP1 (Fig. 4). Both Sp1 sites I and VII were protected to a lesser extent by Sol8 nuclear extracts. We
were unable to identify strong protection of the Ap2 consensus sites; however, Sp1 site VI, which overlaps with one of the Ap2 sites, was weakly protected.

To determine the authenticity of Sp1 protein binding to the Sp1 consensus sequences, DNase I footprint analysis was carried out with purified Sp1 protein. Purified Sp1 protein protected all seven Sp1 binding sites (Fig. 5). Interestingly, the Sp1 sites which are in tandem, II-III and V-VI (Fig. 3), produced an extended footprint, revealing that Sp1 can occupy both sites simultaneously. Taken together, these results demonstrate that Sp1 has the ability to bind to all seven Sp1 sites within the proximal promoter. The variations observed in Sp1 binding patterns may be due to differences in the concentration of Sp1 present in nuclear extracts as compared to purified Sp1 protein. It is also possible that nuclear protein binding to certain sites could affect Sp1 protein binding to sites I and VII.

Mutations to Consensus Sp1 Sites Dramatically Reduced SERCA2 Promoter Activity in Sol8 Cells—To demonstrate which of the Sp1 sites are functionally relevant, consensus Sp1 sites were modified by site-directed mutagenesis. Mutations (GGGCGG → GTTCGG), known to abolish Sp1 binding, were produced for each of the four Sp1 consensus sites (I, III, VI, and VII) individually and in combinations (I+II, I+III+VI) (Fig. 6). SERCA2 –284-CAT constructs containing Sp1 mutations along with a wild type control were transiently transfected into Sol8 cells, and their effects on the promoter function were determined (Fig. 6). Mutation to Sp1 site I or VI reduced the SERCA2 promoter activity by 60–70% as compared to wild type –284-CAT (Fig. 6). Mutation to Sp1 site III or site VII did not significantly alter SERCA2 promoter activity. When mutations were performed on the two distal Sp1 sites I and III in combination, a 53% decrease in reporter activity was observed. Mutations to sites I, III, and VI in combination eliminated 91% of the promoter activity as compared to the wild type construct. These results suggest that multiple Sp1 sites are involved in regulating SERCA2 promoter activity.

Overexpression of Sp1 in Sol8 Muscle Cells Increased SERCA2 Promoter Activity—To determine whether Sp1 can activate the SERCA2 promoter, we cotransfected the SERCA2-promoter constructs (–284-CAT, –267-CAT, and –72-CAT) with the Sp1 expression vector pPACSP1 (25) into Sol8 cells. In response to Sp1 overexpression, the SERCA2 promoter activity was increased significantly (4-fold for –267-CAT and 1.5-fold for –284-CAT) (Fig. 7). Although the –267-CAT SERCA2 construct produces only 40% of maximal CAT activity, overexpression of Sp1 increased the promoter activity (4-fold) to maximal levels comparable to –284-CAT (Fig. 7). The –72-CAT construct, which lacks proximal promoter Sp1 sites, failed to show an increase in CAT activity. In addition, gel mobility shift assays, using a Sp1 consensus oligonucleotide, revealed that the Sp1 protein binding is increased 7-fold in myotubes as a function of Sol8 muscle differentiation (Fig. 8). These results demonstrate that the transcription factor, Sp1, can up-regulate SERCA2 promoter activity, suggesting that the levels of Sp1 can modulate SERCA2 gene transcription in muscle cells.

**DISCUSSION**

In this study we show that the SERCA2 promoter (–1810 bp to +350 bp) contains multiple cis regulatory elements responsible for the complex regulation of this gene in muscle cells. We have identified two positive regulatory regions, a proximal region (–284 bp to –72 bp) and a distal region (–1810 bp to –1110 bp), as important for high level SERCA2 gene expression. In addition, sequences within the –490 bp to –562 bp region had a negative effect on SERCA2 promoter activity. The SERCA2 proximal promoter region (–284 bp to +350 bp) proved to be sufficient for high level expression in Sol8 muscle cells. Interestingly, a short deletion (17 bp) between –284 bp and +350 bp

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**FIG. 7.** Overexpression of Sp1 in Sol8 muscle cells. The Sp1 expression vector pPACSP1 was cotransfected with SERCA2 promoter constructs (–284-CAT, –267-CAT, and –72-CAT) and pBLCAT3 in Sol8 muscle cells. CAT activity is represented relative to the control –284-CAT (no pPACSP1). Data represent the average of at least three experiments.

**FIG. 8.** Sp1 protein binding activity in nuclear extracts of Sol8 myoblasts and myotubes. A double-stranded Sp1 consensus oligonucleotide was end-labeled and incubated without nuclear extract (lane 1), with Sol8 myoblast nuclear extract (lane 2), or with Sol8 myotube nuclear extract (lanes 3 and 4).
and −267 bp produced a significant decrease (69%) in promoter activity, suggesting that this region may contain an important regulatory element. This region (17-bp UPE) was previously shown to bind a 43-kDa protein from C. cebus muscle nuclear extracts (21). However, when the 17-bp UPE was linked to a heterologous promoter, it was unable to promote high level expression,2 suggesting that other regulatory elements within the proximal promoter are required.

Here, we demonstrate that the SERCA2 proximal promoter region (−284 bp to −80 bp) is capable of activating a heterologous (TK) promoter in muscle cells. Interestingly, this DNA fragment functions in a position- and orientation-independent manner, characteristic of a classical enhancer. The proximal promoter region is highly GC-rich and contains seven Sp1 binding sites, which are conserved between the rat and rabbit SERCA2 genes (32).

The results presented in this paper demonstrate that Sp1 plays an important role in the transcriptional regulation of the SERCA2 gene. DNase I footprinting with purified Sp1 protein revealed that all seven Sp1 sites within the proximal promoter can bind Sp1. Nuclear extracts from Sol8 myotubes showed strong protection of Sp1 sites II–VI and weaker protection of sites I and VII. Moreover, site-directed mutagenesis demonstrated that Sp1 binding sites were required for high level expression of the SERCA2 proximal promoter. Furthermore, overexpression of Sp1 in Sol8 muscle cells clearly established that Sp1 can up-regulate SERCA2 promoter activity. Our results can be interpreted to suggest that alterations in Sp1 concentrations can modulate SERCA2 gene transcription.

Although Sp1 is generally regarded as an ubiquitous transcription factor, levels of Sp1 have been shown to vary among different tissues (33). In mouse, Sp1 was shown to be expressed at high levels in tissues undergoing differentiation, and at much lower levels in terminally differentiated cells (33). Our results show that Sp1 binding is increased during Sol8 myogenesis (Fig. 8). Such increases in Sp1 binding could be due to an increase in availability of Sp1 protein or post-translational modifications of the Sp1 protein by phosphorylation or differential glycosylations (34, 35).

It is not, however, surprising that Sp1 overexpression could produce an increase in SERCA2 promoter activity since this promoter contains seven adjacent Sp1 binding sites. Sp1 has been well established as an activator of native (36), as well as artificial (25) promoters that contain multiple Sp1 consensus sites. Sp1, which binds to adjacent sites as seen in the SERCA2 promoter, can interact synergistically to form higher order complexes and activate transcription (30). Our DNase I footprinting data demonstrate that sequences containing adjacent Sp1 sites produce regions of extended protection, suggesting that Sp1 protein can interact to form a higher order complex over this region. Formation of Sp1 complexes are believed to generate an activation environment that allows for more efficient interactions of the transcription apparatus, thus activating transcription at a higher level (30). Transactivation of SERCA2 proximal promoter by Sp1 overexpression provides strong evidence for this type of mechanism.

Although the SERCA2 promoter is expressed at higher levels in Sol8 muscle cells than in NIH 3T3 cells, it is unlikely that muscle specificity is due to the Sp1 transcription factor alone. We propose that the Sp1 protein interacts with other trans acting factors to produce maximal expression of the SERCA2 gene in muscle cells. The −284 bp to −267 bp region, which binds a 43-kDa protein, appears to be essential for the expression of the SERCA2 gene (Ref. 21 and this report). Our DNase I footprinting analysis also revealed a new protein binding site, CATP1, indicating additional trans acting factors might bind within the SERCA2 proximal promoter. However, the relevance of this site in the regulation of the SERCA2 gene remains to be determined. In addition, there are three Ap2 consensus sequences within the proximal promoter that may contribute to the regulation of the SERCA2 gene. Furthermore, the distal promoter region, −1810 bp to −1110 bp, contains consensus sequences for CarG box, E box, and M-CAT elements, which have all been implicated in muscle-specific gene regulation. Our preliminary data indicate that the distal promoter region −1810 bp to −1110 bp can also up-regulate heterologous promoter (TK) expression in muscle cells.2

Interactions between proximal and distal promoter elements may form the basis for the tissue-specific expression of the SERCA2 gene. Sp1 has been shown to be involved in the interactions of proximal and distal enhancers through DNA looping (37). Thus, Sp1 can act as a physical link between proximal and distal promoter activation elements. Sp1 binding in the SERCA2 proximal promoter may play a role in linking distal promoter elements (located in the −1810 bp to −1110 bp) via DNA bending (38) or looping (37), thus providing a better activation environment for the SERCA2 promoter.

Recent studies have also shown that thyroid-responsive elements are located within the proximal promoter of the SERCA2 gene (32, 39, 40). In the rat gene three TRE elements were identified between nucleotides −485 bp and −190 bp (39). In the rabbit gene TRE elements are located between −254 bp and −72 bp (40). Sp1 binding sites were included in the SERCA2 constructs, which gave maximal induction by thyroid hormone. Deletion of the Sp1 binding region decreased T3 inducibility of the promoter (39). Therefore, Sp1 binding sites might be important for thyroid hormone-induced activation of the SERCA2 gene. However, it remains to be determined whether TRE inducibility is dependent on Sp1 interactions with the T3 receptor.

The role of Sp1 in muscle-specific gene expression has been widely established. Muscle-specific genes including cardiac and skeletal α-actin (41, 42) and βMHC (43) require Sp1 for efficient gene expression. Furthermore, the Sp1 transcription factor has been shown to play an important role in muscle-specific gene expression and gene induction by interacting with additional factors that are highly tissue-specific. For example, the cardiac α-actin gene has been shown to require Sp1 in addition to MyoD1 and a SRF-like factor for muscle-specific gene expression (41). Additionally, a Sp1 site was shown to be required along with M-CAT and CarG elements for α1-adrenergic induction of the skeletal α-actin gene (44). The α1-adrenergic inducibility of the ANF gene also appears to be dependent on Sp1 and CarG elements (45). Finally, Sp1 has been shown to regulate gene expression through competition with other factors for binding sites. Sp1 sites within mouse acetylcholinesterase gene proximal promoter region have been shown to overlap with Egr-1 sites resulting in competition between Sp1 and Egr-1 for binding (46). Occupation of the overlapping site by Sp1 up-regulates the acetylcholinesterase gene expression in muscle cells.

In summary, the data presented here suggest that Sp1 plays an essential role in regulating SERCA2 gene expression in muscle cells. We propose that the Sp1 protein may interact with other transcription factors in controlling the efficient expression of this gene in muscle cells. Future experiments will attempt to characterize how the Sp1 binding sites interact with other regulatory elements that are present in the proximal and distal region.

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