Basal Transcription of the Mouse Sarco(endo)plasmic Reticulum Ca\(^{2+}\)-ATPase Type 3 Gene in Endothelial Cells Is Controlled by Ets-1 and Sp1\(^{\ast}\)

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We reported previously that the sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA\(^{1}\)) mediates the active transport of two Ca\(^{2+}\) ions from the cytosol to internal cellular stores after cell activation. SERCA is encoded by a multigene family; three genes, SERCA1 (Atp2a1), SERCA2 (Atp2a2), and SERCA3 (Atp2a3) have been identified, each giving rise to at least two isoforms by alternative splicing (1–3). Two SERCA1 isoforms are produced in a developmentally regulated manner in fast skeletal muscle. The SERCA2 gene gives rise to the ubiquitous SERCA2a isoform and to SERCA2b, which is produced mainly in cardiac, slow skeletal, and smooth muscle cells. SERCA3 is present in specific cell types such as: endothelial cells of a wide variety of tissues (4, 5); epithelial cells of the trachea, salivary glands, and intestine (4, 6); platelets and the hematopoietic cell lineage (7, 8); pancreatic ß-cells and the acini of salivary glands (9); and brain Purkinje cells (6). SERCA3 is expressed early in development, and it is present in the heart tube and in the yolk sac at 10 days post-coitum. At 14 days post-coitum it disappears from the heart tube and is present in the dorsal aorta and as small foci in the liver (10). Several groups (12–15\(^{\S}\))2 have recently identified multiple SERCA3 isoforms generated by alternative splicing, and the respective functions of these isoforms are still unknown.

Disruption of the SERCA3 gene does not critically alter normal mouse development (16). It affects the endothelium- and epithelium-dependent relaxation of smooth muscles suggesting its action on synthesis and/or secretion of vasoactive peptides such as nitric oxide (16, 17). Mutations of the SERCA3 gene have been reported in some forms of type 2 diabetes (18). The structure of the human SERCA3 gene has been documented (14), but data concerning the mechanisms involved in the transcription of this gene remain scarce. There are only few data showing alterations in accumulation of the SERCA3 protein and mRNA. The differentiation of promyelocytic cells along the neutrophil granulocytic pathway induced by all-trans-retinoic acid or CAMP is associated with an increase in SERCA3 and a decrease in SERCA2b transcripts levels, whereas differentiation along the monocyte/macrophage pathway induced by phorbol ester results in an increase in the production of both isoforms (8). In platelets and endothelial cells of spontaneously hypertensive rats, SERCA2b levels are similar to those of normotensive animals, but the SERCA3 isoforms are differentially regulated (19, 20). In the pancreatic islets of non-obese, non-insulin-dependent, and spontaneously diabetic rats (Goto-Kakizaki), the levels of SERCA3 mRNA are 68% lower than in normal rats, whereas SERCA2b production is unaffected (21). Expression of the SERCA3 gene is also reduced during proliferation of endothelial cells in culture (5).

Deletion of the SERCA3 gene results in alteration in one main function of the endothelial cell, i.e. synthesis or secretion of vasoactive peptides such as nitric oxide. Furthermore, in endothelial cells, expression of SERCA3 is regulated in culture and in vivo by an increase in blood pressure. We have therefore undertaken the present study in order to determine the mech-
anisms that regulate the transcription of the SERCA3 gene in endothelial cells. We have identified the transcription start site and showed that one ETS-binding site (EBS) and two Sp1 sites are essential for basal transcription of the gene. We show that the Ets-1 transcription factor is able to transactivate the SERCA3 promoter. Ets-1 is a member of the ETS family, and its expression is correlated to the formation of new blood vessels in the embryo and the adult. Ets-1 is expressed in endothelial precursors during vasculogenesis and in endothelial cells during angiogenesis in the embryo (22). Its pattern of expression is similar to what we showed for SERCA3 during development (10), and Ets-1 is known to transactivate the promoter of several genes that are important in angiogenesis such as the VEGF-R1 and -R2, the Tie receptors, endothelial nitric-oxide synthase, and the VE-cadherin genes (23). We show here that the Ets-1 protein from nuclear extracts of endothelial cells binds to the SERCA3 promoter essential EBS and that Ets-1 is able to promote the transcription of reporter constructs of the gene in endothelial cells. This property is dependent on the integrity of the EBS but also of essential Sp1 sites, suggesting an interaction between the factors on the SERCA3 gene. Furthermore, we show that overexpression of Ets-1 induces the expression of the SERCA3 protein in the cells.

**EXPERIMENTAL PROCEDURES**

**Genomic Cloning and Transcription Start Site Identification**—A 875-bp PstI fragment corresponding to nucleotides (nt) −131 to +744 of the 5′-coding and non-coding region of the rat SERCA3 cDNA clone RK 8−13 (24) was used to screen a mouse (Svj 129) genomic library constructed in the Lambda Fix II vector (Stratagene). Screening of about 20 kb, including exons 1−7 and 8−13, of a genomic clone containing about 20 kb, including exons 1−7 and −8 kb of flanking region of the mouse SERCA3 gene (not shown).

In order to localize the transcription start site, reverse transcription-PCR (RT-PCR) was performed as follows. Total RNA was isolated from mouse tissues or cell lines by the guanidinium thiocyanate procedure (RNA Instapure, Eurogentec). RNA (10 μg) was mixed with 0.2 μg of random hexamer (Boehringer), 10 μg of the gene-specific primer (Invitrogen), 20 μl of a binary mix containing 5′-flanking DNA of total RNA, and the reaction mixture was denatured at 80 °C for 2 min, slowly cooled to 42 °C, and the extension reaction performed for 90 min at 42 °C in the presence of 200 units of Moloney murine leukemia virus reverse transcriptase. The reaction products were precipitated in ethanol and separated by electrophoresis in an 8% polyacrylamide denaturing gel along with a known DNA ladder.

**Cell Culture**—The MoBr 204 cell line derived from mouse brain microvascular endothelial cells was generously provided by Professor P. Vicart (25). EA.hy926 is a hybrid cell line created by fusion of human umbilical vein endothelial cells and a lung carcinoma, A549 cells (26). 3T3-TagEts-1 cells constitutively express mouse Ets-1 following retroviral infection, and 3T3-Neo cells were infected with the corresponding virus to create the reporter cell lines as described by Schreiber et al. (27). For gel shifts, various amounts of proteins, ranging from 2 to 4 μg, were incubated with 0.5 ng of 32P-labeled EBS or Sp1 double-stranded oligonucleotides, in 20 μl of 20 mM HEPES, pH 7.9, 80 mM NaCl, 20 mM KCl, 0.2 mM EDTA, 0.2 mM EGTA, 0.5 mM dithiotreitol, 0.2 mM phenylmethylsulfonyl fluoride, 10% glycerol, and 1 μg of poly(dI-dC) (Amersham Biosciences). In order to establish the specificity of the DNA-protein complexes, purified wild-type and mutated oligonucleotides were used as competitors and were incubated with the proteins for 10 min before addition of the labeled probe. Reaction mixtures were incubated for 30 min at room temperature and were then subjected to electrophoresis in a 6% polyacrylamide non-denaturing gel at 4 °C and 200 V. The sequence of the oligonucleotide probes used (sense strand) is as follows: EBS 5′-GGTCGTATTGCGCGAAATGAGGACGGCA-3′ and Sp1 mut 5′-GGTTGAGCCTAGTTGCCGCAGGTCG-3′, digested with XhoI and BglII, and ligated into the corresponding sites of the −97/+301-Luc construct.

The EBS and Sp1 mutant constructs were generated using the QuickChange™ site-directed mutagenesis kit (Stratagene) using −97/+301-Luc construct as template. The mutations of the mutated EBS oligonucleotides (mut EBS) used are as follows: 5′-GGCTGTATTGCGCGAAATGAGGACGGCA-3′ and 5′-GGCTGTATTGCGCGAAATGAGGACGGCA-3′. The sequence of the oligonucleotide probes used (sense strand) is as follows: EBS 5′-GGTACGTGTA-3′, digested with XhoI and BglII, and ligated into the corresponding sites of the −97/+301-Luc construct.

**Construction of Chimeric Luciferase Expression Vectors**—Various fragments of the SERCA3 promoter all sharing the same 3′ end corresponding to the ATG translation start site were inserted into the pGL3-basic vector (Promega). The −97/+301-Luc construct (+1 correponding to the transcription initiation site) was generated by PCR using the primers 5′-ACCTCTGAGCTCCTAGAGACAGC-3′ and 5′-GCTTCAAGCTTCTGAGCTCCTAGAGACAGC-3′, which anneal into the HindIII restriction site in place of the initial ATG codon. The PCR fragment was digested with XhoI and HindIII and inserted into the pGL3baslic vector. The +153/+301-Luc construct was generated by the Smal digestion of −97/+301-Luc and religation. To generate the −455/+301-Luc construct, a 357-bp XhoI fragment of a genomic clone was added to the −97/+301-Luc construct. The −357/+301-Luc construct was generated by PCR amplification fragment into the −97/+301-Luc construct, using NheI and XhoI sites of the PCR product and the −357/+301-Luc construct. The primers used were 5′-CCTAGCTAGCT- TAGAGATCGGAGTTGA-3′ and 5′-TAGAGATCGGAGTTGA-3′. The −748/+301-Luc construct was produced by subcloning a genomic fragment in the HindIII site of pGL3. To generate the −1341/+301-Luc construct, a fragment was generated by PCR using the primers 5′-CATGCTAGCTCTGGGGT-3′ and 5′-TAGAGATCGGAGTTGA-3′, digested with XhoI and BglII, and ligated into the corresponding sites of the −97/+301-Luc construct.

The EBS and Sp1 mutant constructs were generated using the QuickChange™ site-directed mutagenesis kit (Stratagene) using −97/+301-Luc construct as template. The sequences of the mutated EBS and Sp1 oligonucleotides (mut EBS and mut Sp1) used are as follows: 5′-GGCTGTATTGCGCGAAATGAGGACGGCA-3′ and 5′-GGCTGTATTGCGCGAAATGAGGACGGCA-3′.
thymus, heart, and lung (Fig. 1B), as well as from adult mouse trachea, intestine, and kidney (not shown). This indicated that a single transcription initiation site is located 301 bp upstream from the translation start regardless of the origin of the tissue (hematopoietic, epithelial, and endothelial). This transcription initiation site was referred to as +1 (Fig. 2A).

**Structural Features and Transcriptional Activity of the SERCA3 Promoter**—Although the region located immediately ahead of the identified transcription initiation site showed no consensus TATA or CAAT elements, the non-coding portion of exon 1 was found to be particularly rich in G + C residues (72%) and is most probably responsible for the transcription start (Fig. 2A). Various segments of the promoter region of the SERCA3 gene ranging from −1341 to +301 were cloned ahead of the luciferase reporter gene and analyzed for transcriptional activity in EA.hy926 where SERCA3 is constitutively expressed, or MoBr 204 endothelial cells where the gene is inducible depending on the culture substrates (not shown). The −97/+301 construct displayed a strong activity in both cell lines (31.19 ± 5.3- to 31.2 ± 10.2-fold over 0pGL3 control in EA.hy926 and MoBr 204 cell lines, respectively, \( p < 0.001 \), Fig. 2B), whereas promoter activity progressively decreased when constructs extending further up in the gene. The −337/+301-Luc and −455/+301-Luc constructs showed a significant loss of basal activity in both cell lines, and the −748/+301-Luc construct activity was only 4.35 ± 0.84- and 9.67 ± 4.72-fold that of the 0pGL3 construct in EA.hy926 and MoBr 204, respectively. Finally, the −1341/+301-Luc showed no remaining activity in either cell line. These results demonstrate that the main region responsible for the basal activity of the SERCA3 promoter is located in the −97/+153 fragment of the gene and that negative regulatory elements are located in the region located ahead of nt −455.

**The Sp1 and Ets-1 Elements Are Required for Optimal Transcription of the Mouse SERCA3 Gene**—When comparing the mouse and human gene sequences, a high level of sequence similarity was observed in the proximal non-coding region corresponding to the active promoter region identified above, including the strict conservation of one EBS and two Sp1-binding sites (Fig. 3A). We therefore investigated the possibility that these sites were essential to the transcription of the gene by introducing mutations at either site and testing the transcriptional activity of the resulting construct. Mutation of the EBS resulted in a 70 and 85% loss of activity when transfected in EA.hy926 and MoBr 204 cells, respectively (Fig. 3B), and mutation of the Sp1 sites decreased the transcription activity to ~20% of control values in both cell lines, indicating that the EBS and Sp1 sites were necessary for the basal transcription of the SERCA3 gene. There was no significant difference in transcription activity between EBS and Sp1 mutated constructs.

**The Ets- and Sp1-binding Sites from the SERCA3 Gene Bind the Same Nuclear Factor Complex in Endothelial Cells**—In order to determine whether the EBS and Sp1 sites were able to bind nuclear proteins from endothelial cells, gel mobility shift assays using nuclear extracts from EA.hy926 and MoBr 204 cells were performed. EA.hy926 nuclear proteins formed one major complex with the EBS and Sp1-labeled probes (Fig. 4). Binding to the EBS probe disappeared in the presence of a 100-fold excess of unlabeled wild-type EBS probe but not with unlabeled mutated EBS probe. When testing for binding to the Sp1 probe, complete disappearance of the nucleoprotein complex was observed with the wild-type Sp1 probe. Partial competition was obtained with the unlabeled mutated Sp1 probe, most probably because this probe still contains a partial Sp1 site. The complex observed with the EBS and Sp1 oligonucleotides were of the same molecular weight, suggesting that the
same multimeric complex is bound to both sites. 40 μg of MoBr 204 cell extracts were necessary to observe nucleoprotein complexes, suggesting a lower abundance of the specific nuclear proteins in this cell line.

The proteins that recognized either site were identified by affinity purification using biotinylated probes coupled to agarose beads (Fig. 5). Several members of the Ets family may potentially bind the identified EBS (30). We tested the Ets-1 transcription factor as it is expressed in endothelial cells and associated with the angiogenic phenotype. Western blot analysis of the nuclear proteins bound to the EBS indicated that Ets-1 was present in large amounts in EA.hy926 cells and in lower amounts in MoBr 204 cells. The apparent molecular mass of Ets-1 in the human cell line was 51 kDa, whereas in the mouse it was 62 kDa. In total extract from MoBr 204 cells the two classical Sp1 proteins were observed; the main one corresponded to p95 and a fainter band to p106 (apparent molecular mass of 95 and 106 kDa, respectively). Both proteins were retained on the Sp1 beads. Interestingly, the p106 was also retained on the EBS beads, and significant amounts of Ets-1 were retained by the Sp1 beads. Since the EBS probe does not contain Sp1 sites and reciprocally, this suggests that the Ets-1 and Sp1 factors interact directly with each other on the SERCA3 promoter.

Ets-1 Transactivates the SERCA3 Promoter—Ets-1 was able to transactivate the SERCA3 promoter in MoBr 204 (Fig. 6) as cotransfection of an Ets-1 expression vector increased the activity of the reporter construct by 6-fold. There was no activation of the promoter by Ets-1 which confirms the strong repression observed in this region. There was rather a small repression probably due to transfection of two vectors. Mutation of the identified EBS abolished the activity of Ets-1 on the reporter vector. Interestingly, mutation of the Sp1 site also prevented the activation of the promoter by Ets-1 (3.41 ± 1.23 versus 79.14 ± 25.81), further suggesting that Sp1 and Ets-1 act together for transactivation of the SERCA3 gene promoter.

Ets-1 Induces Expression of SERCA3—In order to verify that expression of the SERCA3 gene was under the control of Ets-1, accumulation of SERCA3 mRNA was analyzed in MoBr 204 cells transfected with either Ets-1-expressing or control vec-
Forty eight hours after transfection, SERCA3 mRNA was detected in Ets-1-transfected cells but not in control cells (Fig. 7A). Two bands corresponding to the alternatively spliced isoforms of SERCA3 (a and b) were detected. On the other hand, the levels of the ubiquitous isoform SERCA2b were similar in both cell types.

In addition, accumulation of the SERCA3 protein was also observed by immunofluorescence 16 h and 3 days after transfection with the Ets-1 expressing vector (Fig. 7B). Large amounts of Ets-1 protein were detected in the nucleus 16 h after transfection (Fig. 7B, a), and accumulation of the SERCA3 protein was detected later (Fig. 7B, e). Similarly, significant amounts of SERCA3 were observed in 3T3-Ets-1 cells that constitutively express Ets-1 (Fig. 7B, g and h) but not in control 3T3-neo cells (Fig. 7B, i). As observed at the transcript levels, there were no significant differences in the levels of the SERCA2b isoform in either cells, indicating that activation of expression by Ets-1 was specific for the SERCA3 isoform (Fig. 7, A and B, c and f).

**DISCUSSION**

We have isolated 20 kb of the mouse SERCA3 gene DNA and shown that transcription was initiated at a unique start site within the TATA-less, GC-rich region located in the first

**Fig. 3.** The Sp1 and EBS elements are required for optimal transcription of the mouse SERCA3 gene. A, alignment of the mouse SERCA3 sequence upstream of the ATG with the human sequence (GenBankTM accession number Y15724) (14). Comparison was performed using LALIGN version 2.0u66 (11). B, MoBr 204 and EA.hy 926 cells were transfected with −97/+301-Luc vector or with the same vector where the EBS or Sp1 sites were mutated (EBS mut and Sp1 mut). Activities obtained with the EBS mut and Sp1 mut constructs are expressed as the percentage of the values obtained with the control −97/+301-Luc construct. The data represent means ± S.D. of three experiments in triplicate. Activities were compared with the −97/+301 construct activity. **, p < 0.01; ***, p < 0.001.
double-stranded oligonucleotides (0.5 ng) were incubated with 5 (EA.hy 926) or 40 μg (MoBr 204) of nuclear extracts in the absence of competitor or in the presence of a 100-fold excess of the wild-type (wt) or mutated (mut) oligonucleotide as competitors. Probe, free probe. One main specific complex formed with either probe (arrow).

**FIG. 4. Electrophoretic mobility shift assays of nuclear extracts from EA.hy 926 and MoBr 204.** 32P-Labeled EBS and Sp1 double-stranded oligonucleotides (0.5 ng) were incubated with 5 (EA.hy 926) or 40 μg (MoBr 204) of nuclear extracts in the absence of competitor or in the presence of a 100-fold excess of the wild-type (wt) or mutated (mut) oligonucleotide as competitors. Probe, free probe. One main specific complex formed with either probe (arrow).

**FIG. 5. Affinity purification of DNA-binding proteins.** 5′-Biotinylated EBS (EBS) or Sp1 (Sp1) double-stranded probes coupled to agarose beads were incubated with nuclear extracts from EA.hy 926 or MoBr 204. Bound proteins were identified by Western blotting using anti-Ets-1 (a-Ets-1) or anti-Sp1 (a-Sp1) antibodies. extract, total cellular extract. Total extract from 3T3-Tag-Ets-1 cells was added as control to the Ets-1 immunoblot.

Exon. SERCA3 reporter gene expression analysis revealed that the basal transcriptional activity of the promoter is contained in the −97/+153 region of the gene, whereas the sequences located upstream act as strong negative regulatory elements. The presence of regulatory regions of transcription located within the first exon is unusual but not unique as it has been reported for other genes expressed in endothelial cells such as von Willebrand factor (31), vascular endothelial growth factor receptor-2 KDR/flk-1 (32, 33), or Tie-2 (34). When comparing within the SERCA family, the unique transcription start site of the mouse SERCA3 detected in RNA from the different tissues is different from that reported for the human gene in tonsil. In this case, transcription was reportedly initiated in a GC-rich region located closer (152 nt) to the AUG translation start codon (14). The human SERCA3 gene lacks TATA or CAAT boxes, as does the mouse gene. The SERCA3 mouse and human genes are, for that matter, different from the two other SERCA genes that possess consensus TATA and CAAT boxes (35, 36).

**FIG. 6. Ets-1 transactivates the SERCA3 promoter.** MoBr 204 cells were transfected with various reporter constructs of the SERCA3 promoter and with either pcDNA Tag-Ets-1 (+) or pcDNA neo (−) as control. Results are expressed as fold induction the activity observed with 0pGL3. The data represent means ± S.D. of three experiments in triplicate. ***, p < 0.001 versus control.

SERCA3 and SERCA2 are coexpressed in various tissues, but they are differentially regulated. Indeed, thyroid hormone increases the transcription of SERCA2, as well as SERCA1, through thyroid hormone-responsive elements (37, 38). However, there is no evidence of a regulation of the SERCA3 gene by thyroid hormone, in accordance with the lack of thyroid hormone-responsive elements in the sequence of the SERCA3 promoter region that we have isolated. Furthermore, we showed here that the SERCA3 promoter was activated by the Ets-1 transcription factor, with a resulting accumulation of SERCA3 mRNA and protein, whereas SERCA2 was not overexpressed in response to Ets-1, either at the RNA or protein levels. On the other hand, we found that Sp1 was essential for the basal transcription of SERCA3, as shown previously (39) for SERCA2 in Sol 8 muscle cells. Thus, although the SERCA genes can be distinctly regulated by specific transcription factors such as Ets-1 or thyroid hormones, they also share common transcriptional activators, such as Sp1. It may well be that the cell-, tissue-, or hormone-specific regulation of the SERCA family depends on the expression of specific coactivators (Ets-1, thyroid hormone) of such common regulators.

Noticeably, SERCA3 mRNA or protein levels were shown to be up-regulated by all-trans-retinoic acid and cAMP (8). Although no consensus binding sites for retinoids are present in the isolated gene fragment, a sequence homologous to CREB was located at −890 which may be responsible for the regulation of SERCA3 by cAMP.

We showed here that the EBS and Sp1 sites, which are conserved between the human and mouse genes, are critical for basal transcription of the SERCA3 gene. This situation is similar to that observed with several endothelial genes where EBS have been shown to be critical for activity of the promoter, such as the mouse VE-cadherin gene (40), Tie-1 (41), Tie-2 (42), Flt-1 (44), and Flk-1 (33), and raised the question of the identification of the ETS factor(s) involved in this regulation. The ETS family contains ~30 transcriptional activators and repressors that share a common DNA binding domain (30). This domain binds to the EBS which is defined as the GGA(A/T) DNA core sequence. Although this core is necessary for binding of the ETS factors, it is absolutely not sufficient for proper recogni-
tion, and the flanking bases play a critical role in the selection of the ETS factor. In the present case, however, the EBS sequence is potentially recognized by most members of the ETS family for which an optimal EBS has been characterized; these include Ets-1, Ets-2, Fli-1, Erg, and other less related ETS factors such as Spi/PU1 or Spi-B (30). However, clues about the ETS factors involved in the regulation of the SERCA3 gene could be found when comparing the embryonic pattern of expression of SERCA3 and certain ETS members. In the embryo, Ets-1 is expressed in the blood islands of the yolk sac where the progenitors of endothelial cells are located and then in endothelial cells of forming blood vessels (43, 44). Ets-1 transcripts are also expressed in mesenchymal cells adjacent to epithelial structures during organogenesis (45, 46). Furthermore, Ets-1 is expressed in the lymphoid organs and in hematopoietic cells (47). This pattern of expression is very similar to that of SERCA3 (3–10). Because functional EBSs had been identified in numerous promoters of genes that are involved in angiogenesis (see above), these observations, taken together, strongly suggested that Ets-1 was a good candidate for regulating the SERCA3 gene in endothelial cells. Indeed, we found that expression of Ets-1 in MoBr 204 or in fibroblasts resulted in the overexpression of the SERCA3 gene. In these experiments, the Ets-1 protein was detected several hours prior to SERCA3 expression, and this is to be expected if the SERCA3 gene is indeed under the control of Ets-1, as the transcription factor has to be present first in order to induce transcription of its target gene. Furthermore, Ets-1 activated the essential EBS found in the SERCA3 promoter, a property lost upon mutation of this EBS, and endothelial nuclear Ets-1 was found to bind the EBS probe used in DNA immunoprecipitation. All these points suggest that Ets-1 participates in the regulation of the SERCA3 gene in endothelial cells. Still, we cannot exclude that other ETS factors do so as well. Erg and Fli-1 are also expressed in endothelial cells during the formation of early endothelial progenitors (48, 49), and Fli-1 is also expressed in hematopoietic lineages (megacaryocytes). Without excluding this possibility, the overall expression patterns of Fli-1 and Erg made them less likely candidates for a control of the SERCA3 gene. The difference in size between Ets-1 in the human and the mouse cell line could be accounted for by differential phosphorylation or by alternative splicing as already reported (50, 51).

Interestingly, we found that mutation of the Sp1-binding sites prevented the activation of transcription of the SERCA3 gene by Ets-1 even in the presence of an intact EBS and that the EBS bound Sp1 proteins and the Sp1 site bound Ets-1. This suggests that Ets-1 and Sp1 control together the expression of SERCA3 and that both factors are most probably present in the same multimeric complex on the SERCA3 promoter. A synergy between Ets and Sp1 was already shown to be important for induction of transcription of tenascin-c (52) and the long terminal repeat of human T cell lymphotrophic virus type 1 (53). This phenomenon might be important for the cell/tissue-regulated expression of SERCA3 evoked above, and Ets-1 could be the endothelial partner of Sp1 for the regulation of the gene in these cells.

Fig. 7. Ets-1 induces the expression of SERCA3. A, RT-PCR analysis of expression of SERCA3 and SERCA2b performed on total RNA from MoBr 204 cells transfected with pcDNA-Tag-Ets-1 (+) or pcDNA-neo (–). B, double immunofluorescence of MoBr 204 transfected with pcDNA-Tag-Ets-1 for 16 (a–c) and 72 h (d–f), or 3T3-Ets-1 (g and h) and 3T3-Neo cells (i). In a, d, and g, cells were labeled with an anti-Ets-1 antibody (green), and in b, e, h, and i they were labeled with an anti-SERCA3 antibody (red). In c and f cells were labeled with an anti-SERCA2b antibody. Bar, 10 μm.
In conclusion, our data demonstrate that Ets factors together with Sp1 are critical to the basal regulation of the SERCA3 gene in endothelial cells. This observation opens the way to the study of a novel role of these factors in the endothelial cell, namely the regulation of the intracellular Ca\(^{2+}\) concentration at the transcriptional level. Furthermore, by regulating many endothelial specific genes, Ets family members are involved in the morphogenetic process essential for capillary formation.

SERCA3 by regulating intracellular Ca\(^{2+}\) may play a fundamental role in this process.

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