Somatostatin Gene Transcription Regulated by a Bipartite Pancreatic Islet D-cell-specific Enhancer Coupled Synergetically to a cAMP Response Element*

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The insulin-, glucagon-, and somatostatin-producing cells in the pancreatic islets derive from a common precursor stem cell and differentiate sequentially during embryonic development, thereby providing an informative model for the study of the transcriptional mechanisms involved in the control of cell-specific gene expression. Relative to the early expression of the glucagon and insulin genes on embryonic days 10 and 12, respectively, the expression of the somatostatin gene is delayed (day 17). The relatively late expression of the somatostatin gene indicates the involvement of both negative and positive transcriptional control mechanisms. We show that the expression of the somatostatin gene in pancreatic islet cells is accomplished by the interplay of both positive and negative cis-regulatory DNA elements. We have characterized the functional properties of one of these positive control elements, the somatostatin gene upstream enhancer element (SMS-UE). The SMS-UE is a pancreatic islet D-cell-specific transcriptional regulator that acts synergistically with the cyclic AMP response element. Mutation-expression and cell-free transcrip- tion analyses show that the SMS-UE is a bipartite element with two interdependent functional domains. Our results indicate that the SMS-UE is part of a functional unit that includes other transcriptional control elements of the somatostatin gene proximal promoter, and that they act together to regulate the D-cell-specific transcription of the somatostatin gene in the islet cells of the pancreas.

The endocrine pancreas provides an informative model in which to investigate the transcriptional mechanisms involved in the control of cell-specific gene expression. It contains a mixed population of cells (islet cells) that synthesize and secrete the peptide hormones glucagon (A-cells), insulin (B-cells), somatostatin (D-cells), and pancreatic polypeptide (F-cells). The phenotypically distinct pancreatic islet cells derive from a common progenitor, probably of endodermal origin, and differentiate sequentially during the course of embryonic development (1-3). Relative to the expression of the glucagon and insulin genes, the onset of the expression of the somatostatin gene is delayed. In the mouse, expression of the somatostatin gene occurs at day 17 of embryonic development in cells that coexpress the insulin gene, which is subsequently repressed in mature somatostatin-producing D-cells. In a different subset of cells that still coexpress both insulin and somatostatin genes, the pancreatic polypeptide gene is activated, and subsequently both insulin and somatostatin genes are repressed (2). This pattern of developmental regulation suggests that the expression of the somatostatin gene is under both positive and negative control mechanisms.

In addition to pancreatic islets, the somatostatin gene is expressed in neurons, C-cells of the thyroid gland, and D-cells of the digestive tract (4). Earlier studies indicated that the expression of the somatostatin gene is modulated by effectors such as cAMP (5, 6), steroid hormones (7), and interleukin-1 (8). Although these effectors probably alter transcriptional mechanisms of control, little is known, with the exception of the cAMP-response element (CRE) (9), about the cis-regulatory sequences that mediate these effects or about the sequences that determine the restricted cellular specificity of the expression of the somatostatin gene. Previous efforts to localize cis-control elements in the 5'-flanking region of the rat somatostatin gene linked to a chloramphenicol acetyltransferase (CAT) reporter plasmid resulted in the identification of a region spanning nucleotides -30 to -60 relative to the transcription start site, that was apparently sufficient to confer cell-specific expression in cells derived from a thyroid medullary carcinoma (10). This region of the somatostatin gene contains the CRE that mediates the transcriptional responses that follow the activation of the cAMP signal transduction pathway (9, 11, 12) via phosphorylation and binding of nuclear factor CREB to the CRE (13). The CRE is recognized by several additional nuclear proteins (14-16) that probably play a key role in the regulation of both the induced and the basal expression of the gene in specific cells.

Binding assays and transient transfection analyses of reporter plasmids bearing regulatory sequences of the glucagon or insulin genes have led to the identification of transcriptional control elements that interact with regulatory proteins to direct pancreatic islet A- or B-cell-specific transcription, respectively. A-cell-specific expression of the glucagon gene is determined by the coordinated activity of at least four different regulatory elements, G1, G2, G3 (17, 18), and a CRE (19). The expression of the insulin gene in B-cells involves

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the binding of a number of nuclear proteins to several cell type-specific cis-acting control elements (20, 21), and a CRE (22), and is also regulated by cAMP (23). Utilizing established cell lines with different hormone-producing phenotypes derived from pancreatic islet tumors, we reported earlier that expression of the somatostatin gene in islet D-cells is regulated by cell-specific positive as well as negative control elements (24). In the present studies reported herein we describe the characterization of a pancreatic D-cell-specific upstream-enhancer element (SMS-UE) located adjacent to the CRE of the somatostatin gene promoter. The SMS-UE is a positive regulator of somatostatin gene expression and acts synergistically with the CRE in both basal and cAMP-induced conditions. By using mutational analyses, we show that the SMS-UE is a bipartite element that contains two distinct but functionally interdependent domains (domains A and B).

EXPERIMENTAL PROCEDURES

Materials—Restriction, ligation, and other DNA-modifying enzymecwere purchased from Pharmacia LKB Biotechnology Inc. Tissue culture media and reagents were obtained from GIBCO-BRL. All other reagents were of molecular biology grade and were obtained from Sigma.

Plasmid Constructs—The plasmids SMS97O and SMS425 were constructed using DNA fragments obtained by polymerase chain reaction amplification of somatostatin gene sequences in the plasmid SMS900 (24). The upstream amplimers were designed to anneal to nucleotide sequences centered on nucleotides -750 or -425 and incorporated a BamHI restriction site in their 5' ends. The downstream amplimers annealed to the sequence corresponding to the XbaI site at position +54. The resulting fragments were digested with the appropriate restriction enzymes, purified on an agarose gel, and ligated into the promoterless plasmid pOCAT (25) that had been digested with BamHI and XbaI. The plasmid SMS425 was constructed by digesting SMS900 with BamHI and KpnI, repairing the ends with T4 DNA polymerase, and religating.

For the construction of SMS120, a synthetic double-stranded oligonucleotide containing the SMS-UE sequence (nucleotides -120 to -69) with BamHI and BglII sites at the 5' and 3' ends, respectively, was ligated into the BamHI site of the plasmid SMS65 (24). The resulting plasmid SMS120 preserves all the somatostatin gene sequences from positions -120 to +54, with the exception of a T to C substitution at position -64. A similar approach was used to construct the plasmids incorporating mutations in the SMS-UE sequence (mutants M1 to M8). The sequences of all the oligonucleotides used for constructing these plasmids are shown in Fig 4A. The construction of the other plasmids used in this study has been described previously (24). The construction of the plasmids used in this study has been described previously (24). The construction of the plasmids used in this study has been described previously (24).

The internal deletion of the CRE in SMS65 or SMS120 was carried out by a stepwise enzymatic procedure. First, the plasmids were digested with the restriction enzyme AatII, which recognizes a 10-bp BamHI site and generates a 3' end. These were digested with T4 DNA polymerase to produce blunt ends. Finally, the plasmids were religated with T4 DNA ligase. The resulting plasmids have a four-base deletion within the core CRE motif (see Fig. 2).

RESULTS

Positive and Negative cis-Regulatory Elements Control the Expression of the Somatostatin Gene—Earlier studies have demonstrated the key role played by the CRE in the regulation of somatostatin gene expression and have also suggested the existence of additional positive and negative cis-regulatory elements located upstream from the CRE. To delineate more precisely the location of these elements, we performed transient transfection assays in pancreatic islet cell lines using CAT reporter plasmids bearing somatostatin gene regulatory sequences. These plasmids were constructed by sequential 5' end deletions of the plasmid SMS900, which contains a fragment of the rat somatostatin gene spanning nucleotides -900 to +54 (24). For transfections we used RIN-1027-B2 cells, derived from a radiation-induced rat pancreatic islet tumor (23). These cells represent the islet D-cell phenotype because they have a high level of somatostatin gene expression and contain only a small fraction of the endogenous insulin gene. We have also shown that these cells express the somatostatin receptor (23).
they express the endogenous somatostatin gene but not detectably the insulin or the glucagon genes (24). The relative level of expression of these fusion genes was compared to that of the Rous sarcoma virus (RSV) enhancer/promoter fused to the CAT-coding sequence. Evidence that the correct transcriptional start site on the somatostatin promoter was utilized was obtained by primer extension analyses carried out on RNA extracted from cells transfected with the somatostatin-CAT fusion plasmids using a 19-base oligonucleotide primer complementary to the coding region of the CAT sequence (17, 18) (data not shown). Most of the fusion gene constructs tested exhibited a level of expression ranging from 15 to 25% of that of RSVCAT, with two exceptions corresponding to the plasmids SMS425 that expressed much less than the other plasmids and SMS120 that expressed at levels comparable to that of RSVCAT (Fig. 1, B and C). Deletions to nucleotide -425 resulted in a significant reduction in CAT activity, indicating the existence of positive regulatory elements located upstream of that position. A further deletion to nucleotide -345 restored the level of expression to about 15% of RSVCAT, suggesting that negative regulatory elements may exist between nucleotides -425 and -345. The activity of the construct in which a deletion to nucleotide -250 was introduced was not significantly different from the construct deleted to nucleotide -345. However, a further deletion to nucleotide -120 resulted in a marked increase in CAT activity (about 120% of RSVCAT), indicating the existence of additional negative regulatory elements located between nucleotides -250 and -120 that have been recently characterized in greater detail. The level of expression of SMS120 was 3-5-fold higher than that of SMS65, which contains the CRE as the only active cis-acting element. We concluded from these experiments that a positive SMS-UE is located between nucleotides -120 and -65 (Fig. 1). The lowest activity in the series of deletion mutated plasmids was observed with SMS42, a minimal promoter plasmid in which the CRE is truncated but that retains the TATA box. The level of expression of SMS42 was similar to that of the promoterless pOCAT plasmid.

The SMS-UE Functions Synergetically with the CRE—The relatively close proximity of the SMS-UE to the CRE suggested the possibility of a functional interaction between the two elements. To test this notion, we investigated whether the positive effect imparted by the SMS-UE was dependent upon the integrity of the CRE. To this end, an internal four-base deletion was introduced into the CRE of both SMS65 and SMS120 to generate the plasmids SMS65ΔCRE and SMS120ΔCRE, respectively (Fig. 2A). Transient transfection assays in RIN-1027-B2 cells indicated that the integrity of the CRE was required for the activity of the SMS65 fusion gene because the level of expression of SMS65ΔCRE was indistinguishable from that observed with the enhancerless SMS42 (Fig. 2B). Deletion of the CRE in the SMS120 construct also resulted in a decrease of CAT activity. However, the level of expression of SMS120ΔCRE was similar to that observed with SMS65, indicating that the SMS-UE is able to support transcriptional activity even in the absence of the CRE. In addition, the activity of SMS120 was 3-5-fold higher than that of either SMS65 or SMS120ΔCRE (Fig. 2B), indicating that both the SMS-UE and the CRE enhance transcription in a synergistic manner.

The above experiments indicate the existence of an interaction between the SMS-UE and the CRE in basal conditions. However, the CRE is essential for the induction of somatostatin gene expression by activation of the cAMP-dependent signal transduction pathway via phosphorylation of transcription factor CREB (13, 34–36). This prompted us to investigate whether a functional interaction between the two cis-regulatory elements also occurs after activation of this second messenger pathway. To test this idea we transfected the pancreatic islet cells of the HIT-T15 line with either SMS65 or SMS120 and treated them with the cAMP analog 8-Br-cAMP (1 mM). HIT-T15 cells were used instead of RIN-1027-B2 cells because it is known that the latter cells have a defective cAMP-induced signal transduction pathway (6, 24), whereas the HIT-T15 cells are responsive to cAMP (24, 37, 38) and contain many of the proteins that bind to the SMS-UE (39). In HIT-T15 cells, the basal level of expression of transfected SMS120 was about 3-fold higher than that of SMS65. Treatment of these cells with 1 mM 8-Br-cAMP for 24 h resulted in a 6.5- and 5.2-fold increase in the CAT activity generated by SMS65 and SMS120, respectively (Fig. 2C). In contrast to what was observed with RIN-1027-B2 cells, the CAT activity of SMS120ΔCRE was close to background levels. The level of expression of SMS120ΔCRE did not increase after 8-Br-cAMP treatment in HIT-T15 cells (Fig. 2C), indicating that the CRE is an essential component.

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2 M. Vallejo, C. P. Miller, and J. F. Habener, manuscript in preparation.
The SMS-UE Is a D-cell-specific Enhancer—It has been shown previously that the SMS900 CAT reporter plasmid is preferentially expressed in islet cells with the D-cell phenotype (24). To determine whether this preferential cell-type expression is also observed with a fragment that contains only the CRE and the SMS-UE, we transfected the SMS120 plasmid into different islet cells and compared its activity with that of RSVCAT. Two cell lines were used in addition to the RIN-1027-B2 cells. One of these, the RIN-1046-38 line, was derived from the same radiation-induced rat pancreatic tumor from which the RIN-1027-B2 cells were obtained, but produces insulin and no somatostatin. RIN-1046-38 therefore represents a B-cell phenotype (3, 24, 26). The other cell line, InR1-G9, is a hamster glucagon-producing cell line that synthesizes no insulin or somatostatin, and thus represents an A-cell phenotype (24, 27). The highest level of expression of SMS120 was observed in RIN-1027-B2 cells and the lowest in RIN-1046-38 cells (Fig. 3A). In the glucagon-producing InR1-G9 cells, the level of expression of SMS120 was about 40% of that observed in RIN-1027-B2 cells. No detectable CAT activity above background levels was observed after transfections with SMS120 in JEG-3 or HeLa cells (not shown). These experiments indicated that a functional unit including the SMS-UE, the CRE, and the TATA box is sufficient for the preferential direction of the expression of the somatostatin gene to islet D-cells.

To investigate whether the SMS-UE is a cell-specific enhancer, three copies of the SMS-UE were cloned in front of the minimal promoter of the herpes simplex virus (HSV) thymidine kinase (TK) gene (40) (−41TKCAT). The design of the SMS-UE oligonucleotides was based on the determination of the exact location of this element by in vitro DNase I footprint assays (see accompanying paper). These constructs were used in transient transfection assays in a variety of islet and non-islet cell lines. Evidence that the TK-CAT mRNA was transcribed from the correct start site was obtained by primer extension analyses of RNA from transfected cells (17, 18). Relative to the activity of pUTKAT, which contains the full TK promoter (25), the level of expression of (SMS-UE)3TK was about 20-fold higher in RIN-1027-B2 cells (Fig. 3B). In all the other cell types tested, in contrast, the level of expression was only 2–3-fold higher than that of pUTKAT. These results indicate that the SMS-UE functions efficiently in somatostatin-producing islet cells, but not in other islet cell types.
and non-islet cells, and therefore is a cell-type-specific regulatory element.

The SMS-UE Contains Two Interdependent Functional Domains—To identify regions within the SMS-UE that are critical for its function, a series of oligonucleotides were prepared which sequentially incorporate four- or five-base mutations spanning the entire element (Fig. 4A). These oligonucleotides were cloned into the BamHI site of SMS65 in a manner analogous to that used to generate SMS120. The resulting plasmids were transfected into RIN-1027-B2 cells, and the CAT activities generated by the plasmids were determined and compared to the activity of the wild type construct, SMS120. Using this approach, two regions of the SMS-UE were identified that are critical for transcriptional activity. The first region, domain A, is located in the 5' region of the element (nucleotides -113 to -107). Mutations in this region (mutants 1 and 2) completely abolished the enhancer activity of the SMS-UE, since the levels of expression of these constructs are similar to that of SMS65 (Fig. 4A). The second region, domain B, spans a broader sequence (mutants 4-7) (Fig. 4A). Mutations in this region also abolished the enhancer activity of the SMS-UE (Fig. 4A). Notably, mutations within the core of this domain (nucleotides -96 to -88, mutants 5 and 6) resulted in a level of expression that was consistently lower than that of SMS65 (Fig. 4A).

In addition to the systematic sequential mutational study, we deleted different portions of the SMS-UE in SMS120 to further assess the functional components of this element. First, we deleted the region corresponding to either the A domain alone or corresponding to both the A and B domains by removing 5' nucleotides to positions -100 or -90, plasmids SMS100 and SMS90, respectively. Second, we removed the B domain and left the A domain by deleting nucleotides -97 to -71, to generate the plasmid SMS120A(97-71). The CAT activity of these constructs was determined after transient transfections in RIN-1027-B2 cells. The level of expression of SMS100 was about 50% less than that of SMS120, but still higher than that of SMS65 (Fig. 4A). In contrast, the level of expression of SMS90 was reproducibly lower than that of SMS65. In addition, the activity of SMS120A(97-71) was also lower than that of SMS65. These results indicate that the integrity of both domains A and B is required for preservation of the functional activity of the SMS-UE. Further, these data suggest that transcription factors that bind to different regions of the SMS-UE may undergo interactions with the CREB or CREB-like CRE-binding proteins located downstream, thereby generating protein complexes that either favor or hamper the transcriptional activity of the gene. If this is true, it could be expected that the precise architecture of the protein complexes that coordinately bind to both the SMS-UE and the CRE are critical for the function of this enhancer unit. To test this hypothesis, we placed an oligonucleotide corresponding to the SMS-UE into the BamHI comparison and is also depicted. CAT activities are expressed as a percentage of that elicited by the wild type SMS120. Panel B, deletion analysis. Relative CAT activities elicited by the SMS120 fusion Gene following transient transfection in RIN-1027-B2 cells. The activity of SMS65 plasmid, which lack the SMS-UE sequence, was measured for comparison.

**Fig. 4. The SMS-UE contains two interdependent functional domains.** Panel A, mutational analysis. The upper panel shows a schematic representation and the relative positions of the A and B domains of the SMS-UE. The shaded area corresponds to the core B domain. The sequences of the wild type and mutant SMS-UE oligonucleotides cloned into the BamHI site of SMS65 are depicted in the middle panel. The mutated nucleotides are double-underlined. The lower panel shows the relative CAT activities generated by the wild type (WT) or mutant (M1-M8) SMS120 fusion gene following transient transfections in RIN-1027-B2 cells. The activity of SMS65 plasmid, which lack the SMS-UE sequence, was measured for comparison.
site of SMS65 in the reverse orientation with the aim to disrupt the spatial distribution of the DNA-binding proteins that recognize these elements. The level of expression of this plasmid in RIN-1027-B2 cells was significantly lower than that of SMS120, and only slightly higher than that of SMS65, indicating the existence of rather rigid spatial constraints between the SMS-UE and the CRE (Fig. 4C).

The introduction of mutations within the SMS-UE may give rise to the creation of unrelated binding sites that are irrelevant to the functions of the element, resulting in the binding of spurious proteins, and the deletional analysis may disrupt the spatial architecture of transcriptionally active complexes. For these reasons, we sought to test the independence of domains A and B by conducting experiments in a cell-free transcription system in conditions in which the SMS-UE is left intact, and transcriptional activity is inhibited by competition of transcription factors by addition of oligonucleotides corresponding to domains A or B of the SMS-UE. For this purpose, we cloned a synthetic oligonucleotide spanning the SMS-UE in front of the somatostatin minimal promoter (SMS42). In the resulting construct, named SMS-UE42, the SMS-UE is placed next to the TATA box. RNA from this plasmid was synthesized in vitro by incubating it with nuclear extracts prepared from RIN-1027-B2 cells. The amounts of correctly initiated transcripts were determined by primer extension analyses using a labeled oligonucleotide that hybridizes to the CAT coding region. The length of the predicted extension products of the hybrid transcripts initiated from the somatostatin promoter combines 45 nucleotides of the CAT gene sequence and 54 nucleotides of the somatostatin gene sequence. No transcripts were detected when the template was incubated in the presence of α-amanitin (1 μg/ml), indicating that the reaction is dependent upon the functional integrity of RNA polymerase II (data not shown). When the SMS-UE42 template was transcribed in the presence of a 100-fold molar excess of an oligonucleotide spanning either the A or B domain of the SMS-UE no transcripts were detected, whereas the addition of equal amounts of an unrelated control oligonucleotide resulted only in a slight decrease in the amount of extension products detected (Fig. 5). These competition experiments in cell-free conditions of transcription indicate that the binding of proteins recognized independently by the A or B domains is required for maintaining the functional activity of the SMS-UE.

**DISCUSSION**

Several studies have been carried out to identify cis-regulatory elements that control the transcriptional activity of the rat somatostatin gene since its structure was first determined (41, 42). Initially, it was found that a short sequence of nucleotides in the 5' flanking region of the gene was required for its specific expression in thyroid medullary carcinoma CA-77 cells (10). This region of the gene also contains the CRE, and therefore mediates the transcriptional responses induced by cAMP (9). The CRE in the context of the immediately adjacent nucleotides binds a number of nuclear proteins (14, 15), one of which is the transcription factor CREB (35, 36). The integrity of this element has been shown to be required for both basal and cAMP-induced expression of the somatostatin gene. A more detailed analysis of this proximal promoter region, led Powers et al. (24) to identify the 5' border of the cAMP responsivity to nucleotide -48, which corresponds to the 5' end of the CRE octamer TGACGTCA, and to establish that the downstream GA-rich region adjacent to the CRE (nucleotides -40 to -30) was also an essential component of this enhancer. Taken together, these observations pointed to the existence of a multiprotein complex that assembles on a region of the somatostatin gene that spans the CRE, the GA box, and the TATA box. This complex is essential for both cell-specific expression and cAMP-induced responses.

A course mapping of the more upstream regions carried out in earlier studies provided evidence for the existence of additional potential cell-specific regulatory elements (24). In the present study, we have carried out a detailed analysis of the 5' flanking region of the somatostatin gene to delineate more precisely the location of transcriptional control elements. Our results indicate that positive regulatory elements exist as far upstream as the region spanning to nucleotide -750. Furthermore, distal and proximal negative control elements are located between positions -425 to -345 and -250 to -120, respectively. A detailed characterization of one of the negative control elements designated as a somatostatin gene proximal silencer element (SMS-PSI), will be reported elsewhere. In addition, our studies reported here indicate the presence of a comparatively active positive regulatory element, the SMS-UE, located between nucleotides -120 and -65. The existence of at least two regulatory elements of opposite action (a silencer and an enhancer) within the region spanning nucleotides -250 to -65 provides an explanation for our earlier findings indicating that 5' deletions to position -250 of the somatostatin fusion gene resulted in transcriptional activities similar to those obtained with deletions to position -65 in somatostatin-producing cells (24). The present findings also provide a mechanistic explanation for the poor transcriptional activity of somatostatin fusion genes with deletions to nucleotide -250 in non-somatostatin expressing islet cells (24), because in the heterologous islet cells, the cell-specific SMS-UE has little or no activity (39), so that the negative influence of the SMS-PSI preempts the positive influence of the SMS-UE.

One alternating purine-pyrimidine sequence of the type d(TG)₅-d(AC)₅ is located between nucleotides -687 and -628 of the 5' flanking region in the rat somatostatin gene (41). Similar sequences in other genes have been found to adopt a Z-DNA conformation (43, 44), and in the prolactin gene they act as silencer sequences that inhibit gene transcription (43).
The results obtained in our studies do not allow us to assign a negative regulatory role to this purine-pyrimidine region in the somatostatin gene because the level of expression of the SMS750 fusion gene was not different from that of the other 5' deletion plasmids tested, with the aforementioned exceptions of SMS425 and SMS120. We cannot rule out, however, that additional positive regulatory control elements located between nucleotides -750 and -425 may compensate for the putative negative effect of the d(TG)-d(AC) sequence. Our findings that multiple regulatory elements modulate the transcriptional activity of the somatostatin gene underscores the complexity of eukaryotic gene regulation and indicate that the concerted action of several DNA elements is required to achieve adequate levels of expression in pancreatic islet cells. The functional interplay between multiple positive and negative elements with different spatial configurations has been found to be important in the regulation of expression of other genes such as growth hormone (45), gastrin (46), immunoglobulin heavy chain enhancer (47), and β-interferon (48).

Experiments in which the CRE was inactivated by an internal deletion demonstrated that the SMS-UE and the CRE act synergistically to enhance transcription in basal as well as in cAMP-induced conditions. Furthermore, we showed that the SMS-UE is able to act independently as a D-cell-specific regulatory element in somatostatin-producing RIN-1027-B2 cells. It therefore appears that the SMS-UE is part of a functional unit, including the CRE, the GA-box, and the TATA box, that is directly involved in determining the cell-specific expression of the somatostatin gene. This notion is supported by the fact that the SMS120 plasmid was expressed only weakly in islet cells with insulin and glucagon phenotypes, but strongly in cells with a somatostatin phenotype, and was not expressed in non-islet HeLa and JEG-3 cells.

The SMS-UE has several properties that are shared by the URE (49) (also referred to as a trophoblast-specific element, TSE) (50), a cell type-specific cis-regulatory element involved in the placental-specific expression of the gene encoding the α subunit of the human chorionic gonadotrophin (αCG). This gene contains two tandem copies of the CRE that act synergetically to maintain basal levels of transcription and to enhance the cAMP-induced responses (51, 52). The CREs themselves confer cell preferential expression to this gene in placental cells (53, 54). Like the SMS-UE, the URE is located upstream from the two CREs of the αCG gene, and acts synergetically with them (55). Both the URE and the CRE combine to form a functional placental cell-specific enhancer (49, 56). The similar αCG URE-CRE and SMS-UE-CRE organization suggests that the cell-specific regulation of these hormone-encoding genes may have similar basic mechanisms of control. An important difference, however, is that the URE is unable to support transcription when cloned in front of a minimal promoter in the absence of a CRE (50, 54), whereas the SMS-UE is still active when the CRE has been deleted or when cloned in front of either the homologous somatostatin or heterologous HSV TK minimal promoters. These observations suggest that the SMS-UE-binding proteins are different from the URE-binding proteins because they have the ability to interact with some component of the basic initiation complex, either directly or through coactivator proteins (56).

Our mutational analysis showed that the SMS-UE is a bipartite element with two functionally interdependent domains. The domain A is located on the 5' end of the SMS-UE, and the domain B is located downstream from A. When isolated from each other, these domains lose their ability to synergize with the CRE; in fact some transcriptionally unfavorable interactions may occur between domain A and the CRE when domain B has been deleted or mutated, as suggested by the diminished CAT activities generated by the plasmids SMS120Δ(97–71) and the SMS120 mutants M5 and M6 following transient transfection in somatostatin-producing islet cells (see Fig. 4). This situation is interesting because the protein that binds the A domain (see accompanying paper) is similar to the CCAAT box-binding factor αCBF, a widely distributed transcription factor which recognizes a cis-regulatory element located closely downstream from the CREs of the αCBF gene (57). It has been suggested that αCBF may be involved in the synergistic interaction that occurs between the two tandem copies of the CRE in this gene (58). This apparent functional discrepancy regarding their interaction with the CRE could be due to the existence of a polarity of the αCBF-CREB-ATF complex, since αCBF is located at opposite extremes of the CRE in the somatostatin and αCG genes. Alternatively, although αCBF and the SMS-UE domain A-binding protein exhibit indistinguishable binding properties (see accompanying paper), it is possible that they are closely related but distinct proteins.

The bipartite nature of the SMS-UE is in contrast with the apparent structure of the related glucagon G3 enhancer (18). Both elements share partial sequence homology and bind similar nuclear proteins (39). Similar to the SMS-UE, the G3 enhancer also has two domains, as defined by in vitro DNA-protein binding assays. However, these domains are not functionally interdependent, so that the modular bipartite structure of the SMS-UE is not shared by the glucagon G3 element. Therefore, despite their similarities, both SMS-UE and G3 are functionally distinct islet cell-specific transcriptional regulatory elements. A key difference between the two elements lies in the fact that the nuclear protein(s) that binds the B domain of the SMS-UE is not present in glucagon-producing cells (accompanying paper). This may account for the functional specificity of these elements observed in somatostatin- and glucagon-producing cells (39).

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