The Proglucagon Gene Upstream Enhancer Contains Positive and Negative Domains Important for Tissue-Specific Proglucagon Gene Transcription

Tianru Jin and Daniel J. Drucker

Department of Medicine, Clinical Biochemistry, and Molecular Genetics
Banting and Best Diabetes Centre
The Toronto Hospital
University of Toronto
Toronto, Ontario, Canada M5G 2C4

The gene encoding proglucagon is restricted in expression to the central nervous system, endocrine pancreas, and intestine. Transgenic experiments indicate that the proglucagon gene upstream enhancer (GUE) element is a principal determinant of both the tissue specificity and the relative level of proglucagon gene transcription. We have now sequenced the rat proglucagon GUE and analyzed the transcriptional properties of proglucagon-luciferase fusion genes (that contain 5′- and 3′-deletions in the GUE) after transfection of islet (InR1-G9) and enteroendocrine (STC-1 and GLUTag) cell lines. The GUE contains both positive and negative elements that are recognized differentially in islet *vs.* intestinal cell lines. The transcriptional properties of the GUE sequences were more similar in cell lines of intestinal (STC-1 and GLUTag) compared with islet (InR1-G9) phenotypes. The electrophoretic mobility shift assay was used to identify specific domains of the GUE that interacted with nuclear proteins from islet and intestinal cells. Several GUE sequences recognized proteins present in both fibroblast and endocrine cell lines. In contrast, electrophoretic mobility shift assay experiments also identified 1) GUE-protein complexes common to both islet and intestinal cell lines and 2) GUE-protein complexes specific to either islet or intestinal lineages. One of the GUE subdomains, designated GLUE1, displayed enhancer-like activity in InR1-G9 and GLUTag, but not BHK, cell lines. Taken together, these observations demonstrate that the proglucagon GUE is comprised of multiple positive and negative domains that likely function in a combinatorial fashion to regulate islet and intestinal-specific proglucagon gene transcription. (Molecular Endocrinology 9: 1306–1320, 1995)

0888-8809/95/$3.00/0
Molecular Endocrinology

INTRODUCTION

The gene encoding proglucagon is expressed in the A cells of the pancreas, selected neurons of the central nervous system, and enteroendocrine cells of the small and large intestine. A single proglucagon mRNA transcript, derived from a common transcription start site, is identical in these tissues; however, cell-specific posttranslational processing gives rise to a different profile of proglucagon-derived peptides in each tissue (1, 2). Initial interest in the biology of islet proglucagon-derived peptide biosynthesis derived from the central role of glucagon, secreted from the pancreas, in the regulation of glucose, fatty acid, and amino acid metabolism (3). More recent studies demonstrating that intestinal-derived glucagon-like peptide 1 (GLP-1) has potent insulinotropic properties (4–6) have also fostered considerable interest in the factors that control the synthesis and secretion of glucagon-like peptide-1 from the intestinal L cell.

Analyses of the control of pancreatic proglucagon gene expression initially used islet cell lines for the elucidation of the signal transduction pathways and transcription factors that regulate proglucagon gene transcription (7, 8). The results of these transfection experiments defined specific regions within the proximal rat proglucagon gene promoter that were important for islet cell-specific proglucagon gene transcription *in vitro* (9). Surprisingly, transgenic mice containing a ~1.3-kilobase fragment of rat proglucagon gene 5′-flanking sequences fused to the coding sequences for SV 40 large T antigen expressed the GLUTag transgene in the brain and pancreas (10), but no transgene expression was observed in the small or

large intestine, suggesting that the molecular determinants that control proglucagon gene transcription are highly tissue specific.

In contrast, a second line of GLUTag transgenic mice containing upstream proglucagon gene sequences (~2.3 kilobases of rat proglucagon gene 5′-flanking sequences fused to the cDNA for SV 40 large T antigen) expressed the transgene in brain, pancreas, and in the endocrine cells of the small and large bowel. Transgene expression in the large bowel of GLUTag(2.4) mice invariably led to cellular hyperplasia followed by the rapid development of proglucagon-producing enteroendocrine tumors *in vivo* (11). Furthermore, GLUTag(2.4) mice consistently developed islet hyperplasia and pancreatic endocrine tumors from 4–8 weeks of age, whereas islet hyperplasia was not detectable until 6 months of age in GLUTag(1.3) mice. These phenotypic differences suggested that the upstream proglucagon gene sequences present in the larger GLUTag(2.4) transgene contain elements important for both islet and intestine-specific proglucagon gene transcription (12).

To elucidate the molecular basis for the differences in transgene expression (and hence phenotype) between the different GLUTag transgenic mice lines, we have initiated studies aimed at characterizing the transcriptional properties of the proglucagon gene sequences upstream of −1300, designated the proglucagon gene upstream enhancer element (GUE). A glucagon-producing cell line arising from the endocrine pancreas and two novel enteroendocrine cell lines from the small and large intestine have been used to examine the cell-specific transcriptional and DNA-binding properties of the GUE *in vitro*. We now report the results of gene transfection and electrophoretic mobility shift assay (EMSA) experiments that identify GUE domains important for both islet and intestinal proglucagon gene transcription.

RESULTS

A series of 5′-and 3′-deletions were created in the upstream rat proglucagon gene 5′-flanking sequences (the GUE as defined in transgenic mice experiments, extends from −2292 to −1253, see Fig. 2). The deletions were sequenced and ligated upstream of the luciferase-coding sequence, and the transcriptional properties of these fusion genes were examined by transfecting three phenotypically distinct rodent cell lines that express the proglucagon gene at high levels: hamster islet InR1-G9 cells, mouse small intestine STC-1, and mouse large intestine GLUTag cells.

The transcriptional activities of the proglucagon-luciferase fusion genes in islet and intestinal cell lines are shown in Fig. 1, a-c. No significant luciferase activity was seen after transfection of the proglucagon gene-luciferase constructs into BHK fibroblasts (Fig. 1d). In contrast, the proglucagon-luciferase plasmids containing the GUE sequences (−1253 to −2292) were much more transcriptionally active in the InR1-G9 islet cell line. The relative luciferase activity observed after transfection of −2292(GLU-LUC) was approximately 150-fold greater than the activity obtained after transfection of the promoterless luciferase plasmid in InR1-G9 islet cells (Fig. 1a). Luciferase activity decreased after deletion of sequences from −2147 to −2058. Further deletion of GUE sequences to −1724 produced a gradual but small (2-fold) increase in relative luciferase activity. In contrast, deletion of GUE sequences from −1561 to −1491 resulted in a marked decrease in luciferase activity (~10-fold), followed by a subsequent increase in luciferase activity after further deletion to −1439, suggesting that the region from −1561 to −1439 contains both positive and negative-acting DNA elements important for transcriptional control in islet cells. Similar to the data obtained with the 5′-deletions, analysis of the transcriptional properties of the 3′-deleted GUE sequences in InR1-G9 islet cells identified both positive and negative elements upstream of −1251.

We next analyzed the transcriptional properties of GUE sequences after transfection of 1) the small intestine-derived STC-1 cell line, and 2) the large intestine-derived GLUTag mouse cell line (Fig. 1, b and c). The relative pattern of GLU-LUC transcriptional activities observed after transfection of STC-1 cells was clearly different compared with the profile obtained after transfection of InR1-G9 cells. The luciferase activity derived from the (−2292)GLU-LUC plasmid (that contains the intact GUE) in STC-1 cells was almost 2-fold greater than the transcriptional activity of the (−1257)GLU-LUC plasmid (Fig. 1b). An increase in luciferase activity was observed after deletion of sequences from −2292 to −2147, and deletion of proglucagon gene sequences from −2,039 to −1,257 resulted in a gradual and progressive (~4-fold) decrease in luciferase activity, suggesting that the GUE contains multiple positive elements differentially recognized by factors in STC-1 compared with InR1-G9 cells. Deletion of GUE 3′-sequences (from −1251 to −1580) resulted in a progressive increase in luciferase activity, and further 3′-deletion to −1683 and −1755 was associated with a decrease in luciferase activity, again consistent with the presence of both positive and negative elements in this region. Deletion of additional sequences to −2050 resulted in an increase in luciferase activity, consistent with the removal of a negative element between −1755 and −2050. It is possible that this negative element extends further 5′- to −2147, as suggested by the data in Fig. 1b-A. Taken together, the transcriptional properties of the proglucagon-Luc plasmids in STC-1 cells are consistent with the presence of both positive and negative elements in the GUE sequence from −1251 to −2292.

Comparison of the transcriptional properties of the GUE sequences in STC-1 small bowel cells compared with the activity of the same plasmids in GLUTag large bowel cells demonstrated considerable similarities between these two intestinal cell lines. Luciferase activity

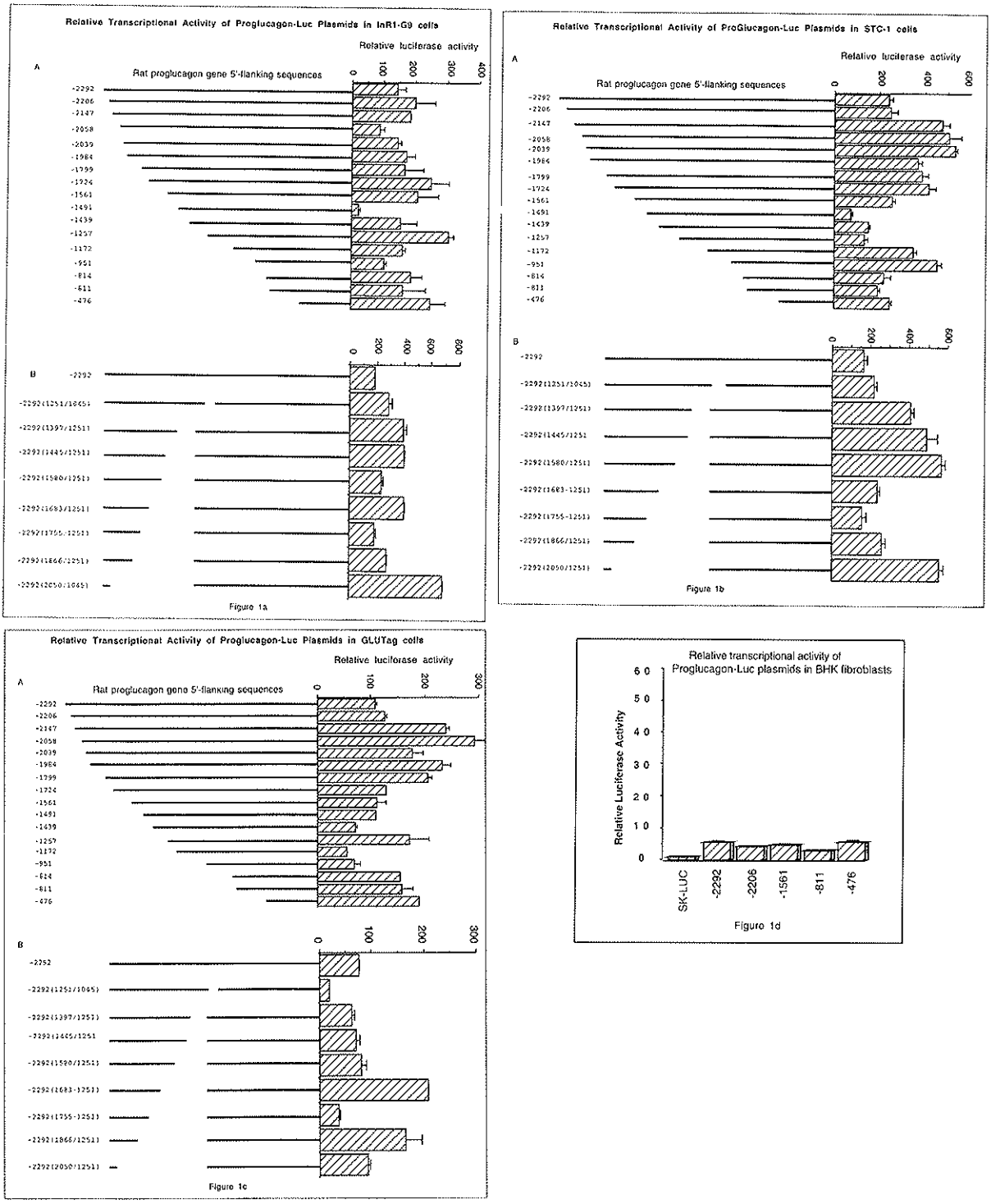

Fig. 1. Transcriptional Activity of the GUE in Pancreatic and Intestinal Cell Lines

a) InR1-G9 cells: 10 μg DNA were transfected by the diethylaminoethyl-Dextran method, and the cells were harvested for analysis of luciferase activity after 18 h. The relative luciferase activity was derived by normalizing values obtained in each assay to the total amount of protein used as well as to the value obtained after transfection of the promoterless luciferase plasmid, SK-LUC, in the same experiment. The data depicted here represent the mean activity ± SEM of three separate transfections (each plasmid was transfected in triplicate for each experiment). For the plasmids shown schematically in panels a-c, the *numbers at the end of the constructs in A* represent the 5′-end of the rat proglucagon gene sequences, whereas the 3′-deleted GLU-LUC plasmids depicted in B extend to nucleotide −2292 at the 5′-end, and the 5′- and 3′-boundaries of the internal deletions are shown in *parentheses*. b) STC-1 cells: 5 μg DNA were transfected into STC-1 cells by electroporation, and the cells were

Fig. 2. DNA Sequence of Rat Proglucagon Gene 5′-Flanking Sequences Including the GUE

An *Eco*RI subclone previously isolated from a rat genomic library containing exon 1 and 5′-flanking sequences was sequenced on both strands, and the sequence was reported, with the first nucleotide of exon 1 designated as +1.

in GLUTag cells rose initially after deletion from −2292 to −2147, then declined progressively after deletion of GUE sequences from −2147 to −1439 (Fig. 1c-A). In contrast to the results obtained with STC-1 cells, deletion of further proglucagon gene sequences from −1439 to −1257 resulted in an increase in luciferase activity in GLUTag cells, consistent with the presence of a negative element in this region.

The transcriptional activity of the 3′-deleted GLU-LUC plasmids was also different in GLUTag cells compared with STC-1 cells (Fig. 1b-B and 1c-B). The luciferase activity of −2292(1251/1045) GLU-LUC was ~25% that obtained after transfection of (−2292)GLU-LUC in GLUTag cells. In contrast, the relative luciferase activity of −2292(1251/1045) GLU-LUC was higher than the activity of −2292(GLU-LUC) in STC-1 cells. Deletion of sequences from −1251 to −1445 resulted in a further increase in luciferase activity in GLUTag cells, consistent with the presence of negative elements in this region, in keeping with the data obtained from transfection of the 5′-deletions (Fig. 1c, A and B). Taken together, the relative transcriptional profiles of proglucagon-luciferase fusion genes containing the GUE sequences derived from transfection of the two intestinal cell lines were similar to each other and clearly different from the data obtained after transfection of the InR1-G9 islet cell line.

The results of the transfection studies suggested that the GUE is comprised of several distinct *cis*-acting sequences that exhibit complex, cell-specific transcriptional properties. The DNA sequences in the rat proglucagon gene promoter extending to −2292 are shown in Fig. 2. The *Kpn*I site, previously mapped to −850 by Efrat *et al.* (10) is actually located at −1253. The cell-specific differences in the transcriptional properties of the proglucagon gene-luciferase fusion genes were most likely attributable to cell-specific expression of DNA-binding proteins that interacted with sequences in the GUE to modulate proglucagon gene transcription. A series of overlapping GUE sequences from −1309 to −2292 was synthesized by polymerase chain reaction (PCR) and used for the analysis of DNA-protein interactions by EMSA. Nuclear extracts were prepared from BHK fibroblasts that

harvested for analysis of luciferase activity after 16 h. The data depicted here represent the mean activity ± SEM of three separate transfections (each plasmid transfected in triplicate in each experiment). c) GLUTag cells: 5 μg DNA were transfected into GLUTag cells by electroporation, and the cells were harvested for analysis of luciferase activity after 24 h. The data depicted here represent the mean activity ± [SEM] of three separate experiments (each plasmid transfected in triplicate for each experiment). d) BHK cells: 10 μg plasmid DNA were transfected by the calcium precipitation method, and the cells were harvested for analysis of luciferase activity after 16 h. The data depicted here represent the mean ± SEM of three separate transfections.

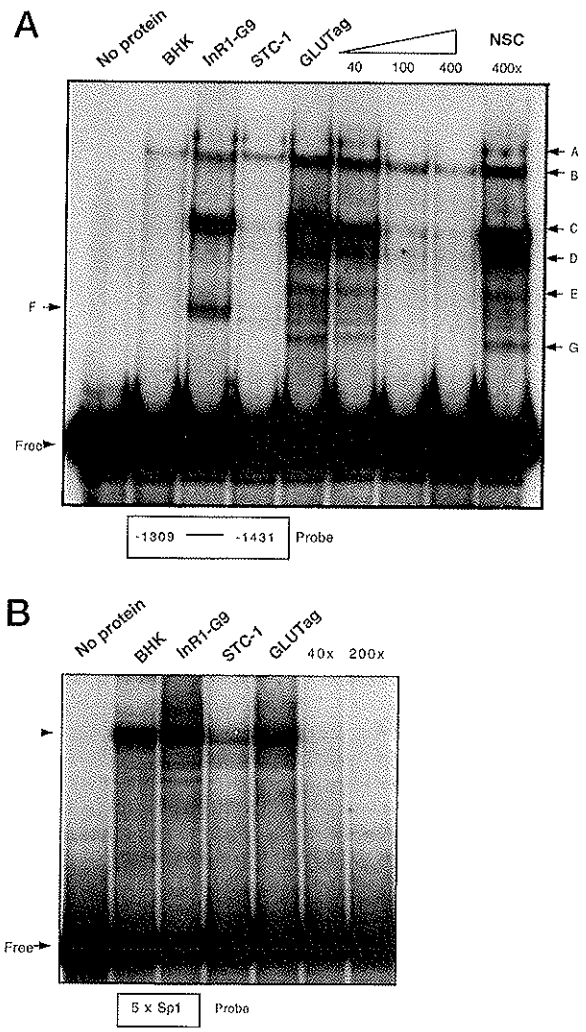

Fig. 3. DNA-Protein Interactions in Glucagon-Producing Cell Lines

A, EMSA using GUE sequences from −1309 to −1431. One microgram of nuclear extract from BHK fibroblasts, InR1-G9 islet cells, STC-1 intestinal cells, and GLUTag intestinal cells was incubated with labeled probe, after which DNA-protein complexes were resolved by polyacrylamide gel electrophoresis and autoradiography. Free = migration position of unbound probe. Specific complexes designated A-G are indicated by *arrows*. Lanes 40, 100, and 400 contain GLUTag nuclear extract incubated with 40- to 400-fold (respectively) excess unlabeled probe in specific competition experiments. The lane marked NSC contains GLUTag nuclear extract incubated with a 400-fold excess of unlabeled nonspecific competitor (−1583/−1462) DNA. B, EMSA using a Sp-1 probe. Four micrograms of nuclear extract from BHK fibroblasts, InR1-G9 islet cells, STC-1 intestinal cells, and GLUTag intestinal cells were incubated with labeled probe, after which DNA-protein complexes were resolved by polyacrylamide gel electrophoresis and autoradiography. Free = migration position of unbound probe. The SP-1-specific complexes are indicated with an *arrowhead*. Lanes 40x and 200x contain GLUTag nuclear extract incubated with 40- to 400-fold (respectively) excess unlabeled probe in specific competition experiments.

did not express the proglucagon gene, as well as the islet and intestinal proglucagon-producing cell lines. Nine unique GUE DNA fragments were generated and used as probes in the EMSA experiments (Figs. 3–12).

The EMSA experiment carried out with the −1309-base pair (bp) to −1431-bp probe is shown in Fig. 3. A number of distinct complexes (designated A-G) were detected that displayed considerable heterogeneity with regard to the cellular specificity of complex formation. Complex B was detected in both fibroblast (BHK), islet (InR1-G9), and intestinal cell lines, and incubation with 400-fold excess specific competitor DNA attenuated the formation of complex B in GLUTag cells (Fig. 3A). In contrast, complex C was quite abundant in nuclear extracts from InR1-G9 and GLUTag cells, barely detected in STC-1 cells, and not detected with BHK nuclear extracts. Furthermore, the formation of complex C using GLUTag extracts was diminished by addition of 40-fold excess specific competitor to the incubation reaction and markedly attenuated with 100-fold excess specific competitor. A third pattern of complex formation, namely GLUTag cell-specific DNA-protein complex formation, was evident in the formation of complexes D, E, and G, which were detected with nuclear extracts from GLUTag cells, but not with extracts from STC-1, InR1-G9, or BHK cells (Fig. 3A). Furthermore, the formation of complexes D, E, and G was effectively diminished in the presence of 100- to 400-fold excess unlabeled probe. Finally, complex F was clearly more abundant in InR1-G9 cells, much less abundant (visible with prolonged exposure of the autoradiographs) in STC-1 and GLUTag cells, and not detected using extracts prepared from BHK cells. In contrast, no competition for specific complex formation was observed in the presence of a 400-fold excess of nonspecific competitor DNA (lane NSC, Fig. 3A).

To determine whether the failure to detect complex formation with specific GUE sequences was attributable to variability in the quality of the different nuclear protein extracts, we carried out an EMSA experiment using the same extracts and a DNA probe containing five copies of a Sp1-binding site. The results of this experiment demonstrated that nuclear extracts from all four cell lines were capable of forming complexes with the Sp1 probe (Fig. 3B), and this binding (in GLUTag cells) was specifically competed by 40-fold excess unlabeled Sp1 probe.

A number of distinct complexes were also detected using GUE sequences from −1387 to −1469 (Fig. 4). Several of the complexes detected with this probe appeared to be similar to those obtained using the −1309 to −1431 probe, likely due to overlapping DNA sequences common to both probes. For example, complexes A, B, C, D, E, and G in Fig. 3 appear to be similar to complexes detected with the −1387/−1469 probe in Fig. 4. In contrast, complex F was detected predominantly in InR1-G9 islet cells as a single band with the −1309/−1431 probe (Fig. 3), whereas a complex designated F observed with the −1387/−1469

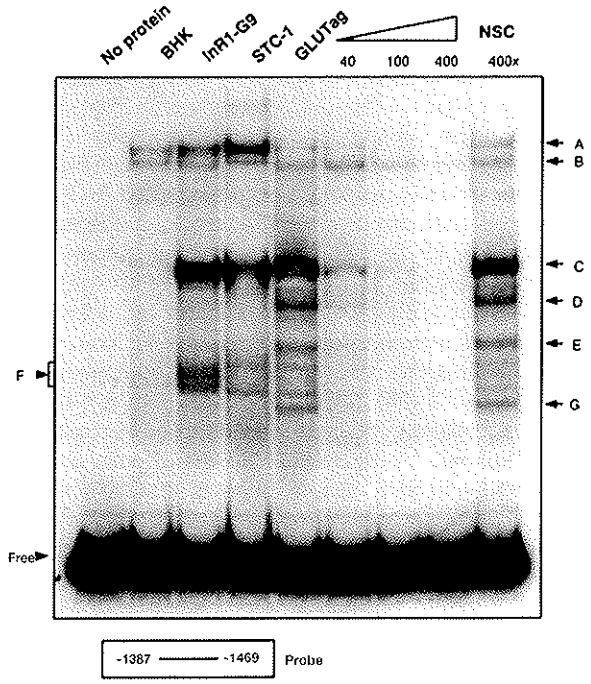

Fig. 4. EMSA Using GUE Sequences from −1387 to −1469

One microgram of nuclear extract from BHK fibroblasts, InR1-G9 islet cells, STC-1 intestinal cells, and GLUTag intestinal cells was incubated with labeled probe, after which DNA-protein complexes were resolved by polyacrylamide gel electrophoresis and autoradiography. Free = migration position of unbound probe. Specific complexes designated A-G are indicated by *arrows*. Lanes 40, 100, and 400 contain GLUTag nuclear extract incubated with 40- to 400-fold (respectively) excess unlabeled probe in specific competition experiments. The lane marked NSC contains GLUTag nuclear extract incubated with a 400-fold excess of unlabeled nonspecific competitor (−1583/−1462) DNA.

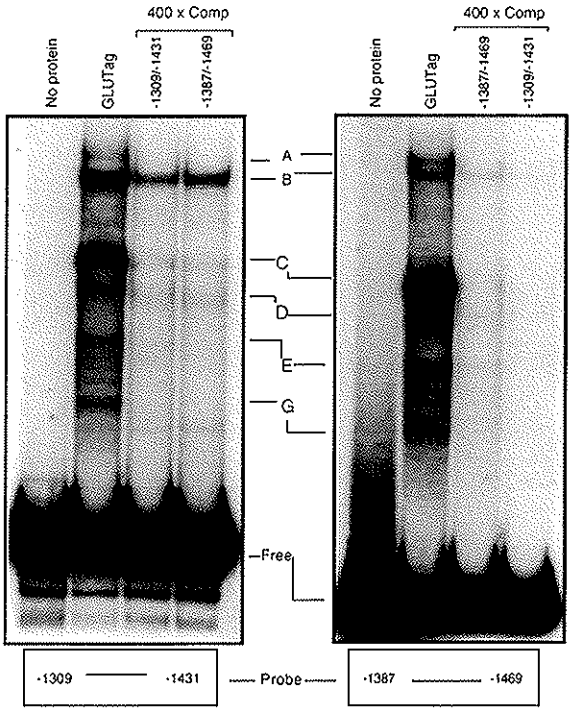

Fig. 5. EMSA Using GUE Sequences from −1309 to −1431 and −1387 to −1469

One microgram of nuclear extract from GLUTag intestinal cells was incubated with labeled probe, after which DNA-protein complexes were resolved by polyacrylamide gel electrophoresis and autoradiography. Free = migration position of unbound probe. Specific complexes designated A-G are indicated by *arrows*. The 1309/−1431 and −1387/−1469 sequences were used in specific competition experiments (400-fold excess).

probe, although of similar mobility to complex F in Fig. 5, migrated as a broader complex containing at least two to three distinct bands (Fig. 4). Since the −1309/−1431 and −1387/−1469 probes contain 45 identical nucleotides, we suspected that many of the similar complexes that migrate in comparable positions in Figs. 3 and 4 were likely attributable to interaction of nuclear proteins with the identical proglucagon gene DNA sequences located from −1387 to −1431. Accordingly, we performed cross-competition experiments with the −1309/−1431 and −1387/−1469 probes and excess unlabeled competitor DNAs, as shown in Fig. 5. The results of this experiment, demonstrating highly efficient cross-competition, suggest that complexes C, D, E, and G (in GLUTag cells, Figs. 3–5) likely arise as a result of the interaction of the proglucagon gene sequences from −1387 to −1431 with GLUTag nuclear proteins.

In contrast to the cell-specific expression of DNA-binding proteins detected with the GUE sequences from −1309 to −1431, sequences from −1462 to −1583 did not result in the formation of cell-specific complexes that could be easily competed by excess unlabeled probe. Complex A was reduced after incubation with 400-fold excess unlabeled specific competitor DNA; however, complex A was also detected with nuclear extracts from fibroblasts and all endocrine cell lines. In contrast, complexes B, D, and E were not diminished by specific competition with excess unlabeled probe (Fig. 6). Similarly, although a number of complexes were detected with BHK, InR1-G9, STC-1, and GLUTag nuclear extracts using a −1582/−1732 probe, these bands were not effectively diminished by excess unlabeled specific competitor DNA (Fig. 7), suggesting that the complexes may result from nonspecific DNA-protein interactions. Alternatively, much higher molar concentrations of specific competitor DNA may be needed to displace the −1582/−1732 probe from complexes A and C. Furthermore, in contrast to the GLUTag-specific complexes detected with various proglucagon gene fragments from −1309 to −1469, no GLUTag-specific complexes were observed with the −1582/−1732 sequences; however, complex B appeared to be unique to the STC-1 cell line.

A number of unique complexes that were eliminated or reduced after competition with excess specific competitor DNA were observed using a −1734/−1866

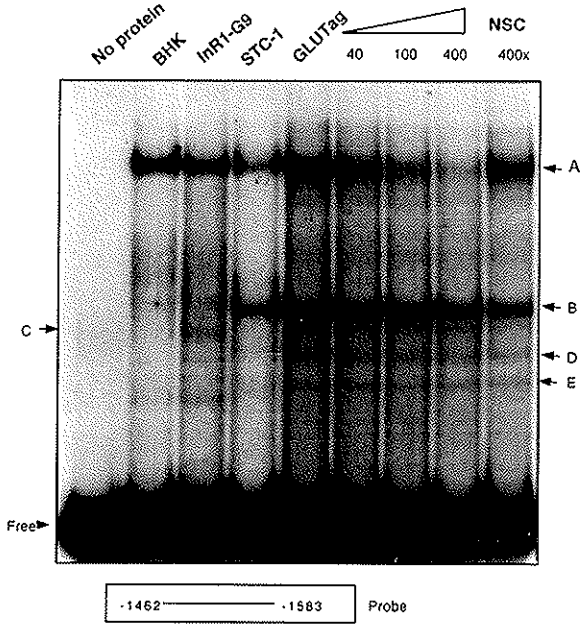

Fig. 6. EMSA Using GUE Sequences from −1462 to −1583

Ten micrograms of nuclear extract from BHK fibroblasts, InR1-G9 islet cells, STC-1 intestinal cells, and GLUTag intestinal cells was incubated with labeled probe, after which DNA-protein complexes were resolved by polyacrylamide gel electrophoresis and autoradiography. Free = migration position of unbound probe. Specific complexes designated A-E are indicated by *arrows*. Lanes 40, 100, and 400 contain GLUTag nuclear extract incubated with 40- to 400-fold (respectively) excess unlabeled probe in specific competition experiments. The lane marked NSC contains GLUTag nuclear extract incubated with a 400-fold excess of unlabeled nonspecific competitor (−1469/−1387) DNA.

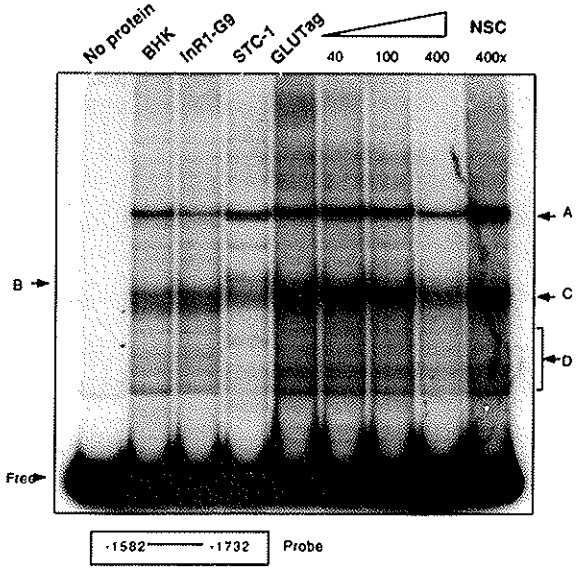

Fig. 7. EMSA Using GUE Sequences from −1582 to −1732

Eight micrograms of nuclear extract from BHK fibroblasts, InR1-G9 islet cells, STC-1 intestinal cells, and GLUTag intestinal cells were incubated with labeled probe, after which DNA-protein complexes were resolved by polyacrylamide gel electrophoresis and autoradiography. Free = migration position of unbound probe. Specific complexes designated A-D are indicated by *arrows*. Lanes 40, 100, and 400 contain GLUTag nuclear extract incubated with 40- to 400-fold (respectively) excess unlabeled probe in specific competition experiments. The lane marked NSC contains GLUTag nuclear extract incubated with a 400-fold excess of unlabeled nonspecific competitor (−1583/−1462) DNA.

probe (Fig. 8). Complex A appeared to represent a slowly migrating band that was detected with nuclear extracts from InR1-G9 cells and GLUTag cells, but not BHK or STC-1 cells. Addition of only 40-fold excess unlabeled specific competitor eliminated the formation of complex A. Complex B was most abundant with BHK nuclear extracts but was also detected with GLUTag extracts; however, the formation of complex B (with GLUTag extracts) was only partially reduced by incubation with 400-fold excess specific competitor DNA. Furthermore, although the complex designated C appeared to be abundant using nuclear extracts from endocrine cell lines, 400-fold excess unlabeled competitor DNA was not very effective in diminishing the formation of this band (using GLUTag extracts), suggesting that complex C may not arise as a consequence of specific DNA-protein interactions, or the binding affinity of proteins present in complex C required higher concentrations of specific competitor for displacement from the −1734/−1866 probe. Two additional complexes, designated D and E, appeared to be predominantly more abundant with GLUTag extracts, yet the formation of complexes D and E was only partially diminished after competition with 400-fold excess specific unlabeled DNA. Similarly, although several distinct complexes were formed using DNA probe −1867/−1996, the formation of only two of these complexes, designated A and D, was diminished after incubation with excess specific competitor DNA (Fig. 9). Incubation of the −1997/−2138 probe with nuclear extracts resulted in the formation of a major complex (A, Fig. 10) that was detected with extract from both fibroblast and endocrine cell lines but was not markedly diminished after incubation with excess specific competitor DNA. Similarly, although the three bands denoted as complex B were also detected with nuclear extracts from all cell lines, only partial attenuation of complex formation was observed with 400-fold excess specific competitor DNA. The −2133/−2292 probe formed three major complexes (bands A, C, and D) with nuclear extracts from InR1-G9 cells that appeared to be distinct from the bands observed with GLUTag nuclear extracts (bands B and E). Nevertheless, band B was somewhat diffuse and was only partially attenuated after incubation with 400-fold specific competitor DNA, whereas the formation of band E was not readily diminished after incubation with excess unlabeled specific competitor DNA (Fig. 11).

To more precisely resolve the DNA-protein interactions observed along a portion of the GUE, we used a smaller (45-bp) probe comprising sequences from

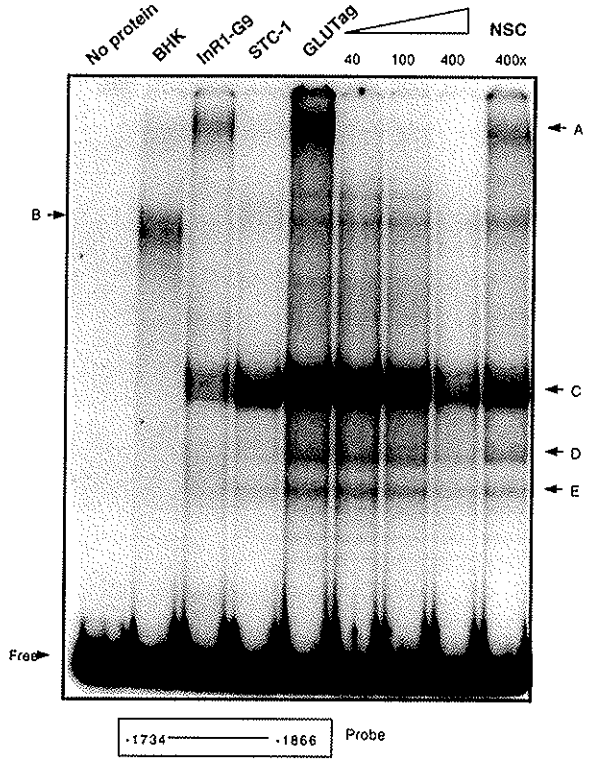

Fig. 8. EMSA Using GUE Sequences from −1734 to −1866
Ten micrograms of nuclear extract from BHK fibroblasts, InR1-G9 islet cells, STC-1 intestinal cells, and GLUTag intestinal cells were incubated with labeled probe, after which DNA-protein complexes were resolved by polyacrylamide gel electrophoresis and autoradiography. Free = migration position of unbound probe. Specific complexes designated A-E are indicated by *arrows*. Lanes 40, 100, and 400 contain GLUTag nuclear extract incubated with 40- to 400-fold (respectively) excess unlabeled probe in specific competition experiments. The lane marked NSC contains GLUTag nuclear extract incubated with a 400-fold excess of unlabeled nonspecific competitor (−1732/−1582) DNA.

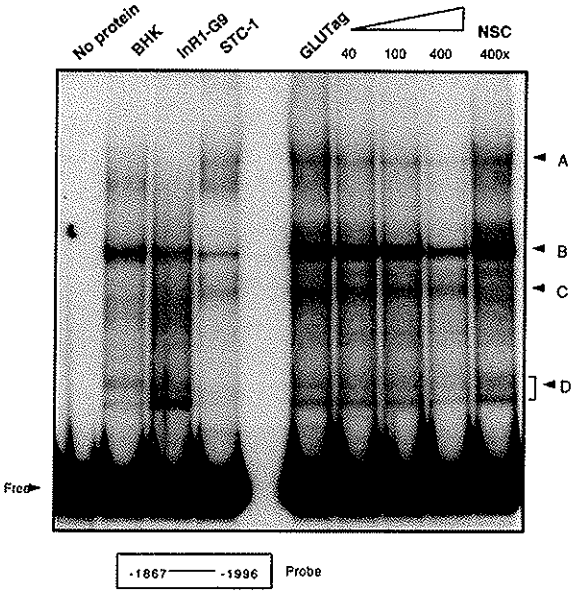

Fig. 9. EMSA Using GUE Sequences from −1867 to −1996
Six micrograms of nuclear extract from BHK fibroblasts, InR1-G9 islet cells, STC-1 intestinal cells, and GLUTag intestinal cells were incubated with labeled probe, after which DNA-protein complexes were resolved by polyacrylamide gel electrophoresis and autoradiography. Free = migration position of unbound probe. Specific complexes designated A-D are indicated by *arrows*. Lanes 40, 100, and 400 contain GLUTag nuclear extract incubated with 40- to 400-fold (respectively) excess unlabeled probe in specific competition experiments. The lane marked NSC contains GLUTag nuclear extract incubated with a 400-fold excess of unlabeled nonspecific competitor (−1866/−1734) DNA.

−1431 to −1387 in EMSA experiments. This specific sequence was selected because the region from −1309 to −1469 appeared to generate the greatest complexity of DNA-protein interactions in EMSA analyses. The results of these experiments using the smaller 45-bp probe are shown in Fig. 12. Nine complexes were observed with the −1431/−1387 probe, using extracts from InR1-G9, STC-1, and GLUTag cells. Complexes A, B, C, H, and I were also detected using BHK cell extracts. Whereas complexes A and B were effectively diminished using only 25-fold molar excess competitor fragment, the intensity of complexes C, H, and I formed with InR1-G9, STC-1, and GLUTag extract was not significantly different after competition with even 100-fold excess competitor DNA. Complexes D and E were detected using both InR1-G9 and GLUTag extract, and these complexes were diminished using only 25-fold molar excess specific competitor fragment. In contrast, complex F was best seen with InR1-G9 cells, whereas complex G appeared to be GLUTag cell-specific, and the formation of both complexes F and G was attenuated by 25-fold molar excess specific competitor. Taken together, these observations provide further evidence for the complexity of DNA-protein interactions along the GUE, even when a smaller (45-bp) subdomain of the proglucagon gene upstream enhancer is used as a probe in EMSA analyses.

To ascertain whether the 45-bp −1431/−1387 sequence (that generates multiple complexes in EMSA experiments) displayed functional properties in proglucagon-producing cells, the 45-bp sequence was inserted, as a single copy, in either the 5′-3′, or 3′-5′ orientation, immediately adjacent to the truncated (−82 to +58)proglucagon promoter in the plasmid pBLUC. The 45-bp element did not augment the relatively low transcriptional activity of the (−82)GLU-LUC plasmid in BHK fibroblasts (Fig. 13A). In contrast, the identical element stimulated (~3.5-fold) the promoter activity of the (−82)GLU-LUC plasmid in InR1-G9 cells, and this enhancer-like activity was seen with the glucagon upstream enhancer 1 (GLUE1) element in an orientation-independent manner. The 45-bp element was also functionally active in GLUTag cells (1.8–2-fold activation) but not in STC-1 cells (Fig. 13). The observation that the GLUE1 element failed to modify the transcriptional activity of the

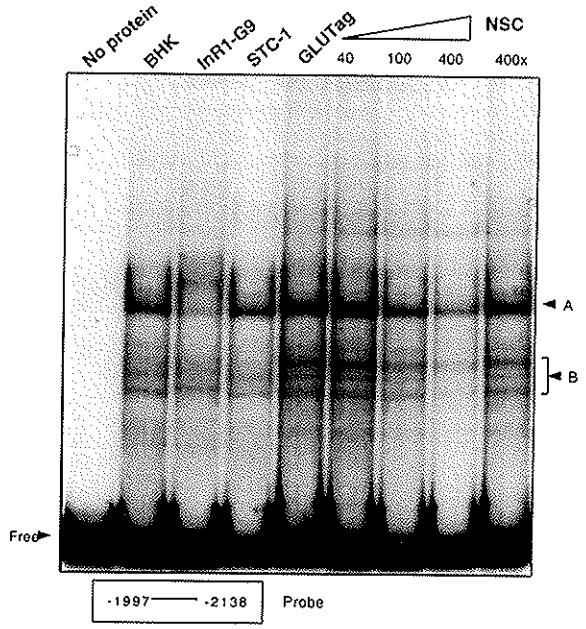

Fig. 10. EMSA Using GUE Sequences from −1997 to −2138

Nuclear extract (4.8 μg) from BHK fibroblasts, InR1-G9 islet cells, STC-1 intestinal cells, and GLUTag intestinal cells was incubated with labeled probe, after which DNA-protein complexes were resolved by polyacrylamide gel electrophoresis and autoradiography. Free = migration position of unbound probe. Specific complexes designated A and B are indicated by *arrows*. Lanes 40, 100, and 400 contain GLUTag nuclear extract incubated with 40- to 400-fold (respectively) excess unlabeled probe in specific competition experiments. The lane marked NSC contains GLUTag nuclear extract incubated with a 400-fold excess of unlabeled nonspecific competitor (−1866/−1734) DNA.

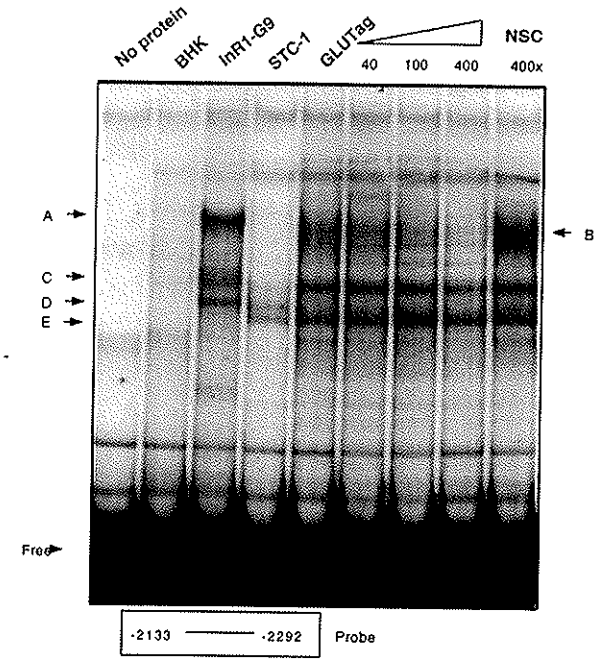

Fig. 11. EMSA Using GUE Sequences from −2133 to −2292

Six micrograms of nuclear extract from BHK fibroblasts, InR1-G9 islet cells, STC-1 intestinal cells, and GLUTag intestinal cells were incubated with labeled probe, after which DNA-protein complexes were resolved by polyacrylamide gel electrophoresis and autoradiography. Free = migration position of unbound probe. Specific complexes designated A-E are indicated by *arrows*. Lanes 40, 100, and 400 contain GLUTag nuclear extract incubated with 40- to 400-fold (respectively) excess unlabeled probe in specific competition experiments. The lane marked NSC contains GLUTag nuclear extract incubated with a 400-fold excess of unlabeled nonspecific competitor (−1866/−1734) DNA.

(−82)GLU-LUC plasmid in STC-1 cells is consistent with the greater complexity of DNA-protein interactions detected with the GLUE1 probe using InR1-G9 and GLUTag extracts (Fig. 12). Taken together, these experiments demonstrate that specific DNA sequences in the proglucagon gene upstream enhancer not only form multiple distinct DNA-protein complexes with extracts from islet and enteroendocrine cells, but a GUE subdomain also displays enhancer-like activity in a cell-specific manner.

DISCUSSION

The results of the transfection and EMSA experiments presented here suggest that multiple *cis*-acting DNA sequences in the GUE contribute to the transcriptional control of proglucagon gene expression in both islet and enteroendocrine cells. We focused our analysis on the GUE due to transgenic experiments suggesting that the presence of the GUE upstream of −1253 produced a phenotype in transgenic experiments consistent with a relatively higher level of transgene expression in the islets (11). Moreover, a GLUTag(1.3) transgene that did not contain the GUE was not expressed in the gastrointestinal tract (10), demonstrating that the GUE is essential for intestinal-specific proglucagon gene transcription *in vivo* (11).

The experiments reported here demonstrate that the GUE contains both positive and negative transcriptional elements in both islet and intestinal cell lines. Although the transcriptional properties of the proximal proglucagon gene promoter have been studied using islet cell lines (8, 9), no reports have previously examined the transcriptional properties of the GUE in islet cells. Furthermore, the data reported here represent the first characterization of the proglucagon promoter and GUE using intestinal cell lines. Although several distinct positive and negative islet cell-specific sequences have been identified in the proximal proglucagon promoter (9, 13), our data demonstrate the existence of both positive and negative elements in the GUE that may be important for proglucagon gene transcription in islets. For example, a major drop in luciferase activity (after transfection of InR1-G9 cells) was observed after deletion of GUE sequences from

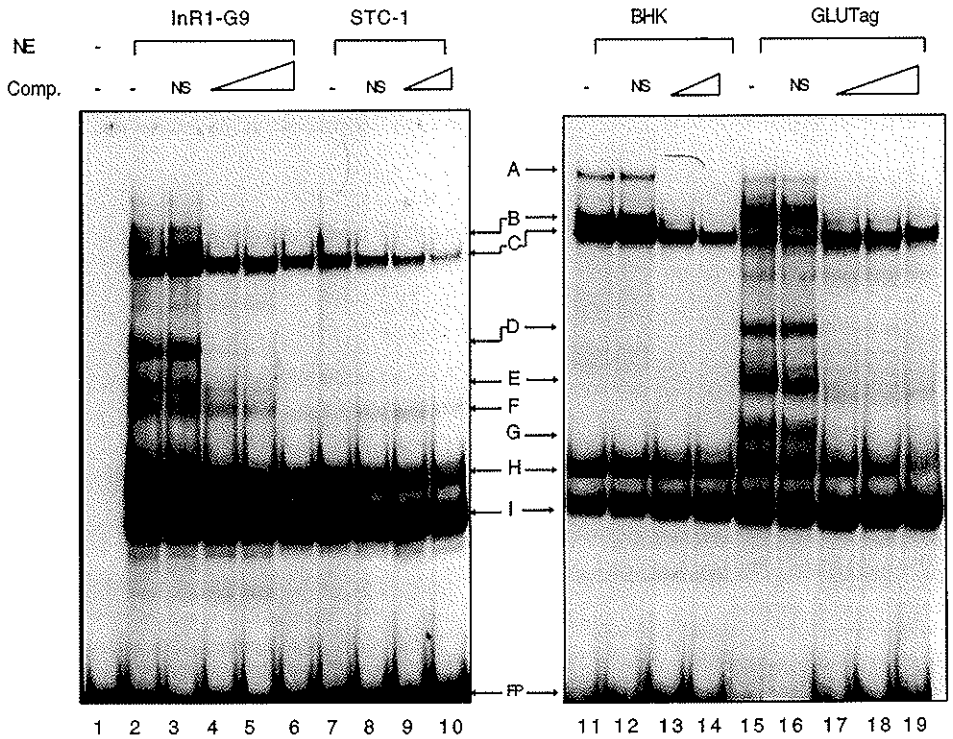

Fig. 12. EMSA Using the Rat Proglucagon Gene Sequences from −1387 to −1431

Five micrograms of nuclear extract (NE) from InR1-G9 islet cells, STC-1 intestinal cells, BHK fibroblast cells, or GLUTag intestinal cells were incubated with labeled probe (0.08 ng DNA, 3×10^4 cpm) in the presence of 2 μg poly dI-dC, after which DNA-protein complexes were resolved by polyacrylamide gel electrophoresis and autoradiography. FP, Migration position of unbound probe. Complexes designated A-I are indicated by *arrows*. The lanes NS contain 100-fold excess unlabeled nonspecific competitor (−1680/−1639) DNA. Increasing amounts of unlabeled specific competitor DNA (25-, 50-, and 100-fold molar excess) were used for InR1-G9 and GLUTag cells; and 50- to 100-fold excess were used for STC-1 and BHK cells to ascertain the binding specificity.

−1561 to −1491. Furthermore, we also identified an InR1-G9-specific complex using DNA sequences from this region (complex C, Fig. 6), raising the possibility that the differential cell-specific activity of the GUE may reflect differences in the expression of cell-specific DNA-binding properties. The importance of these GUE regions for islet cell proglucagon gene transcription may be elucidated by further characterization of the factors that bind to this region, as well as analysis of the functional importance of these sequences for islet proglucagon gene expression *in vivo*.

A number of enteroendocrine-specific complexes were also detected using GUE sequences. The data from the EMSA experiments are summarized in Fig. 14. Unique intestine-specific complexes were more frequently observed using nuclear extracts from the GLUTag cell line, with comparatively fewer complexes observed using extracts from the STC-1 intestinal cell line. Whether this difference reflects the site-specific origin of the two cell lines (large *vs.* small intestine, respectively), or whether the binding events are simply attributable to cell line-specific differences in the relative expression of proglucagon gene transcription factors remains to be determined.

Although the GUE sequences from −1309 to −1469 detected the largest number of DNA-protein complexes, intestine-specific binding events were still detected with more 5′-GUE sequences, including the more distal −2292/−2138 probe (*e.g.* complex E, Fig. 11). Interestingly, we also detected several complexes common to both islet and intestinal cells (Fig. 14), providing evidence that factors common to both islet and intestinal cells may bind to the GUE. Furthermore, the GLUE1 element displayed enhancer-like activity in both islet and intestinal cell lines. These observations provide further evidence suggesting that although GUE sequences are essential for intestine-specific expression, they probably also contribute to regulation of proglucagon gene transcription in the endocrine pancreas.

A number of different experimental approaches have been used for the study of cell-specific gene transcription in the intestine. Analysis of gut development in the nematode *C. elegans* has revealed that multiple positive and negative *cis*-acting elements contribute to intestine-specific expression of the gut-specific ges-1 gene (14). Transgenic experiments employing the fatty acid-binding protein promoter have defined specific spatial and temporal patterns of gene expression in different epithelial cell types of both the small and large intestine in the mouse (15). Similar studies have defined specific DNA elements neces-

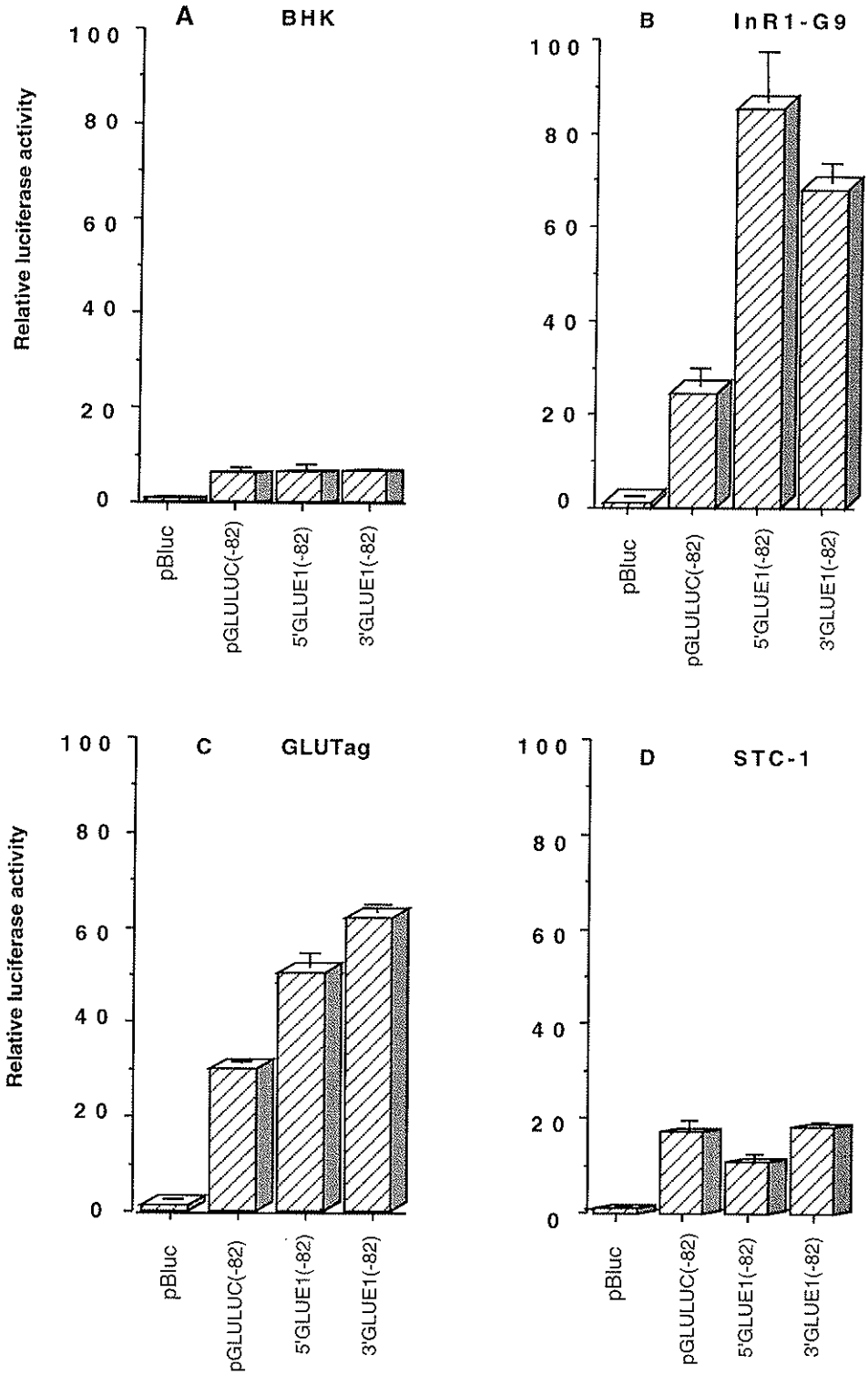

Fig. 13. Transcriptional Properties of the 45-bp (−1431/−1387) Element

pBLUC is a promoterless luciferase reporter gene, and pGlu-LUC(-82) contains the rat proglucagon gene sequences from −82 bp to +58 bp subcloned into pBLUC. The 45-bp sequence was inserted into pGlu-LUC(-82) adjacent to the truncated −82-bp promoter in both the 5′ to 3′, and 3′- to 5′-orientations. Ten micrograms of plasmid DNA were transfected into each cell line, and cells were harvested for analysis of luciferase activity after 16 h. The data depicted here represent the mean ± SEM of three separate transfections.

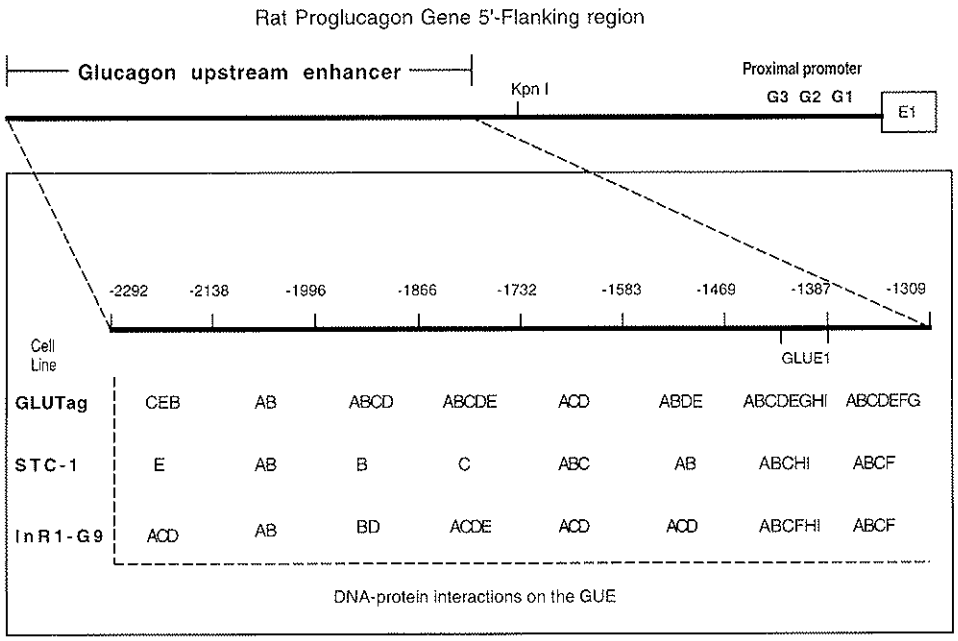

Fig. 14. Summary of DNA-Protein Interactions on the GUE

The complexes [designated A-G, as detected in EMSA experiments (Figs. 3–12)] using specific GUE probes and nuclear extracts from islet (InR1-G9) and intestinal (GLUTag and STC-1) cell lines are represented along the length of the GUE. The nucleotide boundaries of the specific GUE probes are shown. E1 refers to exon 1 and the start of transcription. The proximal promoter elements G1, G2, and G3 have been previously defined (9).

sary for the intestinal-specific expression of the sucrose-isomaltose gene promoter both *in vivo* and *in vitro* (16). These experiments have shown that genes expressed in cell types residing within different regions of the small and large intestine may require different *cis*-acting sequences for correct region-specific expression along the intestine.

A complementary approach for elucidating the molecular determinants of intestinal gene expression involves a more direct transcription factor-based approach. The PCR was used to identify nine different homeobox genes expressed in mouse intestinal epithelium (17). One of the genes identified in this study, designated cdx-2, has subsequently been shown to be expressed specifically in cells of intestinal lineage in a developmentally regulated and region-specific manner (18). More recent experiments have shown that cdx-2 also binds to and activates intestine-specific elements in the sucrase-isomaltase promoter, strongly suggesting that genes encoding cdx-related transcription factors are important determinants of intestine-specific gene transcription (19).

In contrast to our increasing understanding of the transcriptional control of gene expression in nonendocrine cell types of the intestinal epithelium, the mechanisms and regulatory factors responsible for the specification of enteroendocrine-specific gene transcription remain poorly understood. Several different peptide hormone genes are expressed in both the endocrine pancreas and the enteroendocrine cells of the intestine. Genes such as gastrin, peptide YY (PYY), and secretin are expressed only transiently in the fetal endocrine pancreas, after which the pancreas-specific expression is extinguished postnatally (20). In contrast, gastrin, secretin and PYY gene expression continues to be detectable in enteroendocrine cells of adult animals. Although transfection experiments employing islet cell lines have been useful for the examination of tissue-specific regulatory elements important for gastrin and secretin gene transcription (21, 22), the specific DNA sequences and transcription factors that regulate enteroendocrine-specific gene transcription remain unclear.

Both the proglucagon and somatostatin genes are expressed in the endocrine pancreas, nervous system, and intestine. In contrast to the extinguished expression of the gastrin, secretin, and PYY genes in the adult endocrine pancreas, proglucagon and somatostatin gene expression continues at high levels in adult islets. Although considerable information is available about the molecular regulation of somatostatin gene expression in the endocrine pancreas (23, 24), paradoxically little is known about transcriptional control of somatostatin gene expression in the intestine. The paucity of information about the molecular determinants of peptide hormone gene expression in the enteroendocrine cell reflects, in part, the lack of suitable models for carrying out studies of intestinal endocrine gene transcription. The recent isolation and characterization of two novel endocrine cell lines from intestinal tumors (11, 25) has facilitated the analysis presented here of proglucagon gene transcription in cell lines of intestinal origin. STC-1 cells were isolated from aggressive neuroendocrine small bowel tumors

that arose in a single line of double transgenic insulin promoter-SV 40 large T antigen/insulin promoter-polyoma transgenic mice (26). In contrast, the GLUTag cell line was derived from a large bowel endocrine tumor that consistently arises in mice transgenic for a proglucagon-SV 40 T antigen transgene (11). Despite the different sites of origin of these two cell lines, the relative profile of proglucagon-luciferase transcriptional activities was similar after transfection of STC-1 and GLUTag cells. In contrast, the relative transcriptional activities of the identical fusion genes differed considerably after transfection of the InR1-G9 islet cell line. These observations provide further support for previous findings suggesting that the molecular control of proglucagon gene transcription is highly tissue-specific and differs in pancreas and intestine.

Several lines of evidence suggest that the control of gene expression in the pancreas and intestine may be modulated by a number of related transcriptional regulators. Transgenic mice containing a multimerized elastase enhancer element upstream of the human GH reporter expressed the transgene in the pancreas, stomach, and intestine, suggesting that a transcription factor common to these tissues may promote gastroenteropancreatic-specific expression (27). The islets of Langerhans and intestine are both thought to arise from endoderm, suggesting that the similar embryological origin of these two tissues may give rise to a common network of overlapping transcriptional control mechanisms. For example, the somatostatin gene that is expressed in the islets and enteroendocrine cells has been shown to be a target for the homeobox transcription factor IDX-1/IPF-1 (that binds to and transactivates the somatostatin gene promoter) that is expressed in both the islets and intestine (28, 29). Recent studies have also shown that members of the HNF gene family may be important regulators of gene expression in both the intestine and endocrine pancreas (30, 31). Taken together with the observation that a number of GUE-protein binding events appear to be common to cell lines of islet and intestinal origin, the available data support the hypothesis that control of proglucagon gene transcription in pancreas and intestine is likely mediated via a combination of both tissue-specific and shared transcriptional regulatory mechanisms.

Although the proglucagon gene promoter sequences 3′- to −1252 are not sufficient for targeting transgene expression to the intestine *in vivo*, we did not observe major differences in the relative levels of GLU-LUC expression in islet compared with intestinal cell lines. As only a single transgene (containing proglucagon promoter sequences) has been studied (11) that targets expression to the enteroendocrine cell *in vivo*, the precise boundaries of the intestine-specific element that is functional *in vivo* remain unknown. The complexity of DNA-protein interactions along the GUE suggests that it may be useful to carry out additional transgenic studies further delineating the boundaries of the GUE *in vivo*, as such information would greatly facilitate the detailed characterization of the molecular determinants of both islet and enteroendocrine proglucagon gene expression.

MATERIALS AND METHODS

Reagents

Reagents and chemicals were purchased from Sigma (St. Louis, MO), BDH (Toronto, Canada), and ICN Biochemicals (St. Laurent, Canada). Restriction and modification enzymes were products of Boehringer Mannheim (Toronto, Canada). [α-^{32}-P]dATP was obtained from Amersham International (Toronto, Canada). DNA sequencing kits (Sequenase version 2.0) were purchased from U.S. Biochemical (Cleveland, OH). TA cloning kit was the product of Invitrogen (San Diego, CA).

Plasmids

A fragment of the rat proglucagon gene 5′-flanking region (32) from the *Eco*RI site (−2292) to the *Acc*I site in exon 1(+58) was subcloned into the promoterless plasmid SK-Luc immediately adjacent to the coding sequences of the firefly luciferase reporter gene. This plasmid was designated (−2292) GLU-LUC. A series of 5′-deletions (from −2292 to −476 bp) and 3′-internal deletions (from −1041 b.p.) were generated with Exonuclease III. The 5′- and 3′-ends of the various deletions were sequenced to verify the precise nucleotide boundaries. SK-Luc and CMV-Luc were used as negative and positive controls, respectively, in each transfection experiment.

Cell Culture and Transfections

Dulbecco's modified Eagle's medium (DMEM, 4.5 g glucose/liter) was used for growing all the cell lines in this study. The hamster islet cell line InR1-G9 (33) and baby hamster kidney fibroblasts (BHK cells) were grown in DMEM supplemented with 5% calf serum. The mouse small intestinal cell line STC-1 (25) was grown in DMEM supplemented with 2.5% fetal bovine serum and 10% horse serum. The mouse large intestine enteroendocrine cell line GLUTag (34) was grown in DMEM supplemented with 10% fetal bovine serum. InR1-G9 cells were transfected with 10 μg plasmid DNA/10-cm plate by the diethylaminoethyl-dextran method (8). STC-1 cells and GLUTag cells were transfected with 5 μg plasmid DNA/1 $\times$ 10^6 cells by electroporation. Cell extracts were prepared 16–24 h after transfection. For InR1-G9 cells, each set of plasmid transfections was carried out on three separate occasions, with triplicate transfections for each plasmid. For STC-1 and GLUTag cells, each set of plasmid transfections was carried out on four separate occasions, with triplicate transfections for each plasmid. Luciferase activity was analyzed using a LKB-Wallac 1250 luminometer, and relative luciferase activity was normalized to the protein concentration in each extract.

Gel Retardation Assay

Eight probes representing the rat proglucagon DNA sequences from −1309 to −2292 were generated by PCR. The DNA fragments were cloned into a TA vector (Invitrogen), released by *Eco*RI restriction, recovered after electrophoresis on a 12% polyacrylamide gel, and ^{32}-P-labeled with Klenow DNA polymerase. The 45-bp GLUE1 element was generated by annealing two oligonucleotides containing the −1431/−1387 sequence. The EMSA was performed by incubating about 4 $\times$ 10^4 (cpm) end-labeled DNA probe (0.02–0.08 ng

DNA) with nuclear protein (1–10 μg) in binding buffer (10 mM Tris-HCl, pH 8.0, 40 mM KCl, 6% glycerol, 1 mM dithiothreitol, and 0.05% NP-40) for 30 min at 30 C. Each incubation also contained from 2–8 μg poly dI/dC, and the PCR-generated proglucagon gene fragment immediately 5′- or 3′- to the test probe was used as nonspecific competitor DNA. The reaction was then loaded onto a 5% nondenaturing polyacrylamide gel, and after electrophoresis, the gel was exposed to x-ray film for 16–24 h.

The specific primers used to generate the probes used in the EMSA experiments and their corresponding relative locations in the proglucagon gene sequence are as follows. A: −1309/−1431 a)5′ CATGGCTCAGGTCTGGT-3′ and b)5′-CACCAATGAGAAAGTGA-3′; B: −1387/−1469 a)5′GAAGTCACAGCTGTATA-3′ and 5′-ATCTGCCAAGAGCTGCC-3′; C: −1462/−1583 a)5-′TGG CAGATCCAGCAGAT 3′ and b)5′-AGCTCTGCGCTAAGCCA-3′; D: −1582/−1732 a)5′CTCGATTGCTCTTTATG-3′ and b)5′-CTGGTAGTCTAAGAAAA-3′; E: −1734/−1866 a)5′-TTGTGAAGCATCCCTAA-3′ b)5′-ATAGCAGAAGCCAAGTA-3′f)−1867/−1996a)5′-GTTAGAAGTAACCATCC-3′ b)5′-ACA GAA TGC TTC TGG AT 3′; G: −1997/−2138 a)5′-AAAGTTCCCTTCTCTTG-3′ b)5′-GCCAGTGCTAAAGTTCC-3′; H: −2133/−2292 a)5′-ACTGGCCTCTACTTGGC-3′ b)5′-GCTGGAGCTCCACCGCG-3′. The Sp1 probe contained five copies of the Sp1 recognition sequence formed by annealing and ligating two oligonucleotides: a)5′GATCCGGGGCGGGGCGA-3′ and b)5′-GATCTCGCCCCGCCCCG.

Acknowledgment

The authors thank Feng Wang for expert technical assistance.

Received January 24, 1995. Revision received June 7, 1995. Accepted July 10, 1995.

Address requests for reprints to: Dr. D. Drucker, Toronto Hospital- General Division, 200 Elizabeth Street, CCRW3–838, Toronto, Ontario, Canada M5G 2C4.

This work was supported by the Medical Research Council of Canada. DJD is a MRC Scientist, and TJ is supported by a postdoctoral fellowship from the Ontario Ministry of Health.

REFERENCES

1. Orskov C, Holst JJ, Poulsen SS, Kirkegaard P 1987 Pancreatic and intestinal processing of proglucagon in man. Diabetologia 30:874–881
2. Mojsov S, Heinrich G, Wilson IB, Ravazzola M, Orci L, Habener JF 1986 Preproglucagon gene expression in pancreas and intestine diversifies at the level of post-translational processing. J Biol Chem 261: 11880–11889
3. Unger RH 1985 Glucagon physiology and pathophysiology in the light of new advances. Diabetologia 28:574–578
4. Mojsov S, Weir GC, Habener JF 1987 Insulinotropin: Glucagon-like peptide I (7–37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. J Clin Invest 79:616–619
5. Kreymann B, Ghatei MA, Williams G, Bloom SR 1987 Glucagon-like peptide-1 7–36: A physiological incretin in man. Lancet 2:1300–1304
6. Holst JJ, Orskov C, Nielsen OV, Schwartz TW 1987 Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. FEBS Lett 211:169–174
7. Philippe J, Drucker DJ, Habener JF 1987 Glucagon gene transcription in an islet cell line is regulated via a protein kinase C-activated pathway. J Biol Chem 262:1823–1828
8. Drucker DJ, Philippe J, Jepeal L, Habener JF 1987 Glucagon gene 5′-flanking sequences promote islet cell-specific glucagon gene transcription. J Biol Chem 262: 15659–15665
9. Philippe J, Drucker DJ, Knepel W, Jepeal L, Misulovin Z, Habener JF 1988 Alpha-cell-specific expression of the glucagon gene is conferred to the glucagon promoter element by the interactions of DNA-binding proteins. Mol Cell Biol 8:4877–4888
10. Efrat S, Teitelman G, Anwar M, Ruggiero D, Hanahan D 1988 Glucagon gene regulatory region directs oncoprotein expression to neurons and pancreatic alpha cells. Neuron 1:605–613
11. Lee YC, Asa SL, Drucker DJ 1992 Glucagon gene 5′-flanking sequences direct expression of SV40 large T antigen to the intestine producing carcinoma of the large bowel in transgenic mice. J Biol Chem 267: 10705–10708
12. Drucker DJ 1994 Molecular pathophysiology of glucagon-SV40 T antigen transgenic mice. Am J Physiol Endocrinol Metab 267:E629–E635
13. Knepel W, Jepeal L, Habener JF 1990 A pancreatic islet cell-specific enhancer-like element in the glucagon gene contains two domains binding distinct cellular proteins. J Biol Chem 265:8725–8735
14. Aamodt EJ, Chung MA, McGhee JD 1991 Spatial control of gut-specific gene expression during *Caenorhabditis elegans* development. Science 252:579–582
15. Cohn SM, Roth KA, Birkenmeier EH, Gordon JI 1991 Temporal and spatial patterns of transgene expression in aging adult mice provide insights about the origins, organization and differentiation of the intestinal epithelium. Proc Natl Acad Sci USA 88:1034–1038
16. Wu GD, Wang W, Traber PG 1992 Isolation and characterization of the human sucrase-isomaltase gene and demonstration of intestine-specific transcriptional elements. J Biol Chem 267:7863–7870
17. James R, Kazenwadel J 1991 Homeobox gene expression in the intestinal epithelium of adult mice. J Biol Chem 266:3246–3251
18. James R, Erler T, Kazenwadel J 1994 Structure of the murine homeobox gene cdx-2. J Biol Chem 269:15229–15237
19. Suh E, Chen L, Taylor J, Traber PG 1994 A homeodomain protein related to caudal regulates intestine-specific gene transcription. Mol Cell Biol 14:7340–7351
20. Brand SJ, Fuller PJ 1988 Differential gastrin gene expression in rat gastrointestinal tract and pancreas during neonatal development. J Biol Chem 263:5341–5347
21. Wheeler MB, Nishitani J, Buchan AMJ, Kopin AS, Chey WY, Chang T-M, Leiter AB 1992 Identification of a transcriptional enhancer important for enteroendocrine and pancreatic islet cell-specific expression of the secretin gene. Mol Cell Biol 12:3531–3539 s
22. Brand SJ, Wang TC 1988 Gastrin gene expression and regulation in rat islet cell lines. J Biol Chem 263:16597–16603
23. Powers AC, Tedeschi F, Wright KE, Chan JS, Habener JF 1989 Somatostatin gene expression in pancreatic islet cells is directed by cell-specific DNA control elements and DNA-binding proteins. J Biol Chem 264:10048–10056
24. Vallejo M, Penchuk L, Habener JF 1992 Somatostatin gene upstream enhancer element activated by a protein complex consisting of CREB, Isl-1-like, and a-CBF-like transcription factors. J Biol Chem 267: 12876–12884
25. Rindi G, Grant SGN, Yiangou Y, Ghatei MA, Bloom SR, Bautch VL, Solcia E, Polak JM 1990 Development of neuroendocrine tumors in the gastrointestinal tract of transgenic mice. Am J Pathol 136:1349–1363

26. Grant SGN, Seidman I, Hanahan D, Bautch VL 1991 Early invasiveness characterizes metastatic carcinoid tumors in transgenic mice. Cancer Res 51:4917–4923
27. Rose SD, Kruse F, Swift GH, MacDonald RJ, Hammer RE 1994 A single element of the elastase I enhancer is sufficient to direct transcription selectively to the pancreas and gut. Mol Cell Biol 14:2048–2057
28. Leonard J, Peers B, Johnson T, Ferrei K, Lee S, Montminy MR 1993 Characterization of somatostatin transactivating factor-1, a novel homeobox factor that stimulates somatostatin gene expression in pancreatic islet cells. Mol Endocrinol 7:1275–1283
29. Miller CP, McGehee Jr RE, Habener JF, Moseley JM, Chou ST, Martin TJ 1994 IDX-1:a new homeodomain transcription factor expressed in rat pancreatic islets and duodenum that transactivates the somatostatin gene. EMBO J 13:1145–1156
30. Wu GD, Chen L, Forslund K, Traber PG 1994 Hepatocyte nuclear factor-p1a(HNF-1a) and HNF-1b regulate transcription via two elements in an intestine-specific promoter. J Biol Chem 269:17080–17085
31. Philippe J, Morel C, Prezioso VR 1994 Glucagon gene expression is negatively regulated by hepatocyte nuclear factor 3b. Mol Cell Biol 14:3514–3523
32. Heinrich G, Gros P, Habener JF 1984 Glucagon gene sequence:four of the six exons encode separate functional domains of rat pre-proglucagon. J Biol Chem 259: 14082–14087
33. Drucker DJ, Philippe J, Mojsov S 1988 Proglucagon gene expression and posttranslational processing in a hamster islet cell line. Endocrinology 123:1861–1867
34. Drucker DJ, Jin T, Asa SL, Young TA, Brubaker PL 1994 Activation of proglucagon gene transcription by protein kinase A in a novel mouse enteroendocrine cell line. Mol Endocrinol 8:1646–1655