Identification of cis- and trans-Active Factors Regulating Human Islet Amyloid Polypeptide Gene Expression in Pancreatic β-Cells*

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Iset amyloid polypeptide is expressed almost exclusively in pancreatic β- and δ-cells. Here we report that β-cell-specific expression of the human islet amyloid polypeptide gene is principally regulated by promoter proximal sequences. The sequences that control tissue-specific expression were mapped between nucleotides −2798 and +450 of the human islet amyloid polypeptide (IAPP) gene for transgenic mice. To localize the cis-acting elements involved in this response, we examined the effects of mutations within these sequences using transfected islet amyloid polypeptide promoter expression constructs in pancreatic β cell lines. The sequences between −222 and +450 bp were found to be necessary for β cell-specific expression. Linker-scanning mutations of the 5′-promoter proximal region defined several key distinct control elements, including a negative-acting element at −111/−102 base pairs (bp), positive-acting elements like the basic helix-loop-helix-like binding site at −135/−131 bp, and the three A/T-rich, homeobox-like sites at −172/−163, −154/−142, and −91/−84 bp. Mutations within any one of these elements eliminated transcriptional expression by the promoter. Gel mobility shift assays revealed that the PDX-1 homeobox factor, which is required for insulin gene transcription in β cells, interacted specifically at the −154/−142 and −91/−84 bp sites. Since PDX-1 is highly enriched in β and δ cells, these results suggest that this factor plays a principal role in defining islet β cell- and δ cell-specific expression of the IAPP gene.

Amyloid deposits are a common feature in individuals with non-insulin-dependent diabetes mellitus (NIDDM)1 (1). Islet amyloid polypeptide (IAPP) or amylin, which is a member of the calcitonin gene family (2), is the most abundant component of pancreatic amyloid. This 37-amino acid peptide is normally secreted with insulin from β cells (3) and is also expressed in a subset of islet δ cells (4).

The physiological role IAPP plays normally or in NIDDM is unclear. As a consequence of its abilities to inhibit insulin secretion in isolated islets (5, 6) and to counteract insulin action in peripheral tissues (7), IAPP has been proposed to play an important role in regulating plasma glucose levels in mammals. The formation of diabetes-associated amyloid deposits appears to be related to the primary sequence of IAPP, as only certain species, which include primates and cats, encode a hydrophobic amyloidogenic core of amino acids (amino acids 20–29) that allow fibril formation (8). However, since amyloid deposits are not normally found in nondiabetic individuals, other factors must also contribute to disease formation. It has been proposed that elevated expression of IAPP may be one such factor (9).

Experiments conducted by German et al. (10) indicate that transcription of the IAPP gene is controlled by a factor that is also involved in β cell-specific expression of the insulin gene. Thus, it was demonstrated that the −176 to −117-bp region of the human IAPP gene could direct cell-specific transcription from a heterologous promoter in transient transfection assays with characteristics similar to the −247 to −197-bp region of rat insulin I gene. This region of the insulin gene I is regulated by A/T-rich elements and a basic helix-loop-helix (B-HLH) factor binding site (10, 11). The regulatory factors that bind to and activate the A/T-rich elements appear to be shared between the IAPP and insulin genes (10).

The PDX-1 homeoprotein, which is selectively expressed in islet β and δ cells as well as specific enteroendocrine cell-types in the duodenum (12), activates expression from A/T-rich elements of the insulin gene (13–16). Interestingly, homozygous PDX-1 mutant mice fail to form a pancreas (17, 18), and the enteroendocrine cells in the duodenum are also affected (18). Recently, two laboratories have identified factors that appear to regulate expression from the promoter proximal A/T-rich elements in the IAPP gene. However, the conclusions drawn from these studies are quite different. Bretherton-Watt et al. (19) concluded that PDX-1 interacts with these A/T-rich elements in β cells, whereas Wang and Drucker (20) demonstrated that a distinct factor, the LIM homeodomain protein Isl-1, can activate IAPP expression.

In the present study, we have examined the effects of mutations throughout the human IAPP gene on β cell-type-specific expression. This resulted in the identification of a minimal control region spanning sequences from −222 to +450 bp, which was regulated by a number of distinct cis-acting elements. The positive control elements included the A/T-rich elements at −172/−163, −154/−142, and −91/−84 bp and a B-HLH-like binding site at −138/−131. In addition, this analysis identified a negative-acting element at −111/−102 bp. Using the gel shift assay, we found that PDX-1 composed the major β-cell binding activity with the human IAPP elements at −154/−142 and −91/−84 bp. In contrast, Isl-1 did not appear to bind to these elements. These results indicate that there are several distinct factors acting upon the sequences within the

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¶ The abbreviations used are: NIDDM, non-insulin-dependent diabetes mellitus; IAPP, islet amyloid polypeptide; LUC, luciferase; CAT, chloramphenicol acetyl transferase; RSV, Rous sarcoma virus, bp, base pair(s); B-HLH, basic helix-loop-helix; GH, growth hormone; PCR, polymerase chain reaction; EF-1, elongation factor 1.
5'-flanking region of the IAPP gene to control islet β cell-specific expression. Furthermore, one of these factors, PDX-1, is common to the insulin gene.

**EXPERIMENTAL PROCEDURES**

**Generation of Transgenic Mice and Immunohistochemical Staining**—The IAPP-GH fusion gene was constructed from human IAPP and growth hormone (GH) sequences using standard cloning techniques. IAPP sequences were isolated from the luciferase reporter plasmid pSLA12 (21) and spanned human IAPP sequences from 241 by deleting the amylin intron 1. The oligonucleotide PCR primers were flanking end point. The exon I internal deletion mutants were constructed in pAm-222 by a modification of the procedure of Gustin and Burke (24). The IAPP promoter mutant oligos used in the PCR reactions are listed in Fig. 5.

**Plasmid Constructions**—All of the IAPP promoter mutants were cloned into the chloramphenicol acetyltransferase (CAT) expression vector, pTC, which is a Bluescript II SK plasmid (Stratagene) containing the CAT reporter gene fused to β-globin poly(A) sequences. Each of the clonal internal deletion mutants were generated in pAm-391 by standard molecular techniques. The intron 1 deletion mutant, pAm-241 (Δ1) and pAm-434, was constructed in pAm-241 by deleting the amylin intron 1. The oligonucleotide PCR primers spanned human IAPP sequences from 241 to 231 (5'-AGATCTGAG-GCAAATTC-3') and bridged exon 1 and exon 2 (5'-CATTAAAAA-GAAAAATTGGAGAGCAGGT-3'). The IAPP-human elongation factor-1 α (EF-1) intron mutants were generated by cloning EF-1 intron 2 (+1726 to +2091) and EF-1 intron 4 (+2674 to +2756) fragments generated by the PCR reaction between the IAPP +103 (exon 1) and +436 (exon 2) sequences in pAm-391 to yield pAm-391EF-1Int2 and pAm-391EF-1Int4. The IAPP promoter linker-scanning mutants were constructed in pAm-222 by a modification of the procedure of Gustin and Burke (24). The IAPP promoter mutant oligos used in the PCR reactions are listed in Fig. 5.

**Cell Culture and Transient Transfections**—βTC3 (kindly provided by Shimon Efrat, Albert Einstein College of Medicine, Bronx, NY), RINm5F (from ATCC), and HeLa S3 cells were maintained in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. All expression constructs were introduced into cells by electroporation with the CellPorator (Life Technologies, Inc., Gaithersburg, MD) using conditions described previously (25). IAPP-CAT-human elongation factor-1 α (EF-1) intron 2 was co-transfected along with 2 μg of RSV-long terminal repeat promoter luciferase (LUC) expression plasmid, RSV-LUC; the LUC activity from RSV-LUC was used to control for transfection efficiency. Expression from the IAPP-CAT constructs was compared with a herpes simplex virus thymidine kinase promoter-driven CAT reporter construct, pBLCAT2 (26), transfected in parallel. LUC and CAT activities were measured from 10 μg of crude extract 40–48 h after transfection. The protein concentration of the crude lysate was determined by the method of Bradford (27). CAT and LUC activities were determined using the methods described by Kingston and Sheen (28). Each experiment was repeated a minimum of 3 times.

**Western Blot Analysis**—Nuclear extracts from βTC3 cells and human adult islets were prepared as described (29). βTC3 (0.5–10 μg) and islet (10 and 20 μg) extract protein were resolved on a 12% SDS-PAGE and electrophoresed to an Immobilon polyvinylidene fluoride membrane (Millipore, Bedford, MA). The membranes were either probed with N-terminal PDX-1 or Isl-1 K5 (31) polyclonal antisera (1:500 dilution in a buffer composed of 5% nonfat dry milk in Tris-buffered saline (140 mM NaCl, 10 mM Tris-HCL, pH 7.5) + 0.5% Tween 20). The N-terminal XHbox8 antisera was developed against the first 75 amino acids of the Xenopus XHbox8 protein and cross-reacts with the mouse PDX-1 protein (14); and the Isl-1 K-5 antisera was generated against C-terminal residues 178–349 of rat Isl-1 (31). The blot was washed and probed with peroxidase-conjugated protein A as described previously (14). The positions of the bound antibodies were detected by autoluminography with the ECL detection kit (Amersham Life Science Inc., Arlington Heights, IL).

**Electrophoretic Mobility Shift Assays**—Nuclear extract (5 μg) from HeLa and βTC3 cell lines and human islet extracts were used in gel shift assays with double-stranded 32P-labeled human IAPP oligonucleotides probes (A1, 32PATGGAAATATGAGAGG; A2, 32ACT-GATGAGTTAATGAAATGACC; A3, 32ATTGGTCTAGTTATCTCT). The positions of the bound antibodies were detected by autoradiography with the ECL detection kit (Amersham Life Science Inc., Arlington Heights, IL).

**Nuclear Immunoblot Analysis**—Transgenic IAPP-GH mouse pancreas cryosections were immunostained with a mouse antiserum to human IAPP (A1), which was raised against the C-terminal residues 178–349 of rat Isl-1 (31). The blot was washed and probed with peroxidase-conjugated protein A as described previously (14). The positions of the bound antibodies were detected by autoluminography with the ECL detection kit (Amersham Life Science Inc., Arlington Heights, IL).

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and extract. The samples were analyzed on a 6% nondenaturing polyacrylamide gel run at 4 °C in TGE buffer (50 mM Tris, 380 mM glycine, and 2 mM EDTA, pH 8.5). After electrophoresis, the gel was dried and subjected to autoradiography.

RESULTS

**IAPP Promoter Activity in Transgenic Mice**—To localize the sequences of the human IAPP gene that were sufficient for islet-specific expression, a 3.3-kilobase region of the genomic clone l201 (32) was ligated to the human GH gene and expression examined in transgenic mice. The IAPP sequences contained within the IAPP-GH reporter included 2798 bp of 5' flanking sequence, exon 1 (102 bp), intron 1 (333 bp), and a portion of exon 2 (15 bp). There were no IAPP protein coding sequences present in the GH reporter construct. IAPP-GH was subjected to pronuclear microinjection to generate six founder lines, one of which (line HGC) was shown by Northern analysis to express the transgene. To determine whether the transgene was expressed within islets, we prepared serial sections from the pancreas of transgenic and nontransgenic littermates and examined them for GH and insulin expression immunohistochemically (Fig. 1). We detected GH staining within the islets of transgenic mice but not their nontransgenic littermates. In contrast, insulin expression was observed in the islets of both sets of animals. The co-incidence of GH and insulin staining indicates that the majority of human GH-containing cells were β cells. These results are consistent with the expression pattern for the endogenous IAPP protein (4). In contrast, GH staining was not detected in any other tissues tested, including kidney, liver, spleen, stomach, skin, muscle, and brain. These results indicated that the region from −2798 to +450 bp was sufficient to direct islet-specific expression of the IAPP gene.

**Identification of 5' and 3' Flanking Sequences that Regulate IAPP Expression in β Cells**—As a first step toward identifying the IAPP sequences within the 2798/1450 bp control region that were necessary for islet β cell expression, we subcloned a series of IAPP control region mutants into a bacterial CAT expression plasmid. The CAT activities of the wild-type and mutant IAPP-CAT constructs in βTC-3 (B), RIN-m5F (C), and HeLa S3 cells (D). The results represent the normalized mean percent CAT acetylation (% CAT) from at least three independent transfections ± S.D.

![Figure 2](http://www.jbc.org/)

**Fig. 2. Effect of exon 1 mutagenesis on pAm-391 activity.** The exon 1 mutants were constructed within pAm-391 as described under "Experimental Procedures." A, exon 1 sequences within wild-type (pAm-391) and mutant constructs. The transcriptional activities of the wild-type and mutant IAPP-CAT constructs in βTC-3 (B), RIN-m5F (C), and HeLa S3 cells (D). The results indicate that the region from −2798 to +450 bp was sufficient to direct islet-specific expression of the IAPP gene.

2 W. C. Soeller, P. Roche, and P. C. Butler, unpublished results.
To determine the exon 1 and intron 1 sequences that were important in expression, internal deletion mutants within these regions were constructed in the context of human IAPP sequences spanning 2391/1450 (Fig. 2) or 2241/1450 (Fig. 3). There was only a small change in IAPP-CAT reporter gene activity as a consequence of this 5′ deletion (compare pAm-391 with pAm-222 in Fig. 4). All of the exon 1 deletion mutants were active in the transfected β cell lines and inactive in HeLa cells (Figs. 2 and 3; data not shown). The activity of the largest exon 1 deletion mutant, which spanned sequences from 15 to 147 bp, was reduced approximately 2.8-fold when compared with pAm-391 (Fig. 2, B and C). In contrast, precise deletion of intron 1 from pAm-241 resulted in a 20-fold drop in activity (Fig. 3A). To determine whether the loss in pAm-241Δ104–434 activity resulted from the removal of essential cis-active transcriptional control sequences, we replaced the IAPP intron with nonrelated intron sequences from the generally expressed elongation factor 1 (EF-1) gene (33). The activity of the constructs containing either EF-1 intron 2 or EF-1 intron 4 sequences were very similar to the IAPP wild-type construct (compare pAm-391 with either pAm-391EF-1Int2 or pAm-391EF-1Int4 in Fig. 3B). Together, these results indicate that IAPP transgene transcription within β cells was not profoundly affected by the human IAPP noncoding sequences present in the construct. However, our results suggest that a post-transcriptional processing event may have been very important in the expression of IAPP-GH. We therefore have retained the IAPP noncoding sequences to +450bp within each of the 5′-flanking control region mutants constructs in the studies described below.

To identify the 5′-flanking region sequences within the human IAPP promoter that were important in β-cell expression, we generated a series of 5′-flanking IAPP-CAT deletion constructs. The expression pattern obtained with these mutants was similar between the β cell lines, βTC3 and RIN-m5F (Fig. 4, A and B). Thus, the chimera with only 222 bp of upstream sequence, pAm-222, had approximately the same activity as the −2798 construct, pAm-2798. In contrast, deletion of the IAPP sequences between −222 to −138 resulted in a 6–10-fold drop in activity in both β cell lines. This region contains the A/T-rich motifs that appear to be functionally similar to those of the insulin gene (10). The generally lower activity of the IAPP-CAT construct in RIN-m5F cells, when compared with βTC-3, is also reflected in their IAPP and insulin levels (21, 42). All of the 5′-deletion constructs were inactive in HeLa cells (Fig. 4D). These studies indicate that the sequences between −222 to +450 bp are responsible for directing IAPP gene expression to β cells.

Identification of cis-Acting Elements Involved in Regulating β Cell-specific Expression—To identify the IAPP control elements within the 5′ proximal region that were important in β cell-type-specific activity, we constructed a series of 10-bp linker-scanner mutants between positions −211 and −32 bp in pAm-222 (Fig. 5). This strategy allowed us to analyze the importance of small regions of the IAPP gene in transcriptional control without altering the spacing between putative promoter elements. The activity of each construct was assayed in βTC3 and RIN-m5F cells.

There were several IAPP promoter sequences sensitive to
mutation in transfected β cells (Fig. 5). In some instances, we observed that mutant IAPP-CAT activity was reduced primarily in only one of the β cell lines. In this category were the mutants at −201/−192, and −81/−71 bp. We presume that their activators were only limiting for IAPP-CAT expression in the sensitive β cell line. We believe that the more interesting mutants were those that influenced IAPP-CAT activity in both RIN-m5F and βTC-3 cells as these probably represent the action of regulators that are essential for transcription. The phenotype of these mutants indicates that IAPP gene expression is regulated by both positive- and negative-acting cellular activities. Thus mutations within activator sites, like the A/T-rich elements at −172/−163, −154/−142, and −91/−84 bp or the B-HLH-like site at −138/−131, reduced activity by 65–75% (compare the activity of the C, D, E, F, G, and L mutants with pAm-222 in Fig. 5A). In accordance with the nomenclature used in naming the A/T-rich and B-HLH sites in the insulin gene (34), the A/T-rich elements at −172/−163, −154/−142, and −91/−84 bp will be referred to as A3, A2, and A1, respectively, and the B-HLH-like site as E1. Each of the A and E element mutants profoundly affected pAm-222 activity, implying that the activators functioning at these sites act cooperatively to mediate IAPP expression. In contrast to the A/T-rich and B-HLH-like site mutants, the properties of −111/−102 mutant indicates that it is a repressor binding site. This result does not appear to be caused by artifactual generation of an activator binding site since the 2–8-fold increase in activity observed with the −111/−102 site mutant was also obtained with two other linker-scanner mutants over this region.³

³ M. D. Carty, J. S. Lillquist, and W. C. Soeller, unpublished results.

PDX-1 Is the Predominant β Cell Activity Binding to the A1 and A2 Elements—The gel mobility shift assay was used to determine the distribution of the cellular factors interacting with the A1, A2, and A3 elements. Nuclear extracts were prepared from βTC3 and HeLa S3 cells. Binding reactions were conducted in the presence of extract and ³²P-labeled human IAPP A-element probes spanning sequences at −183/−161 (A3), −163/−138 (A2), and −96/−76 (A1) bp. Binding to these IAPP elements was compared with the insulin A2 element binding site at −213 to −192 bp, which interacts with the PDX-1 transcription factor (14). Several protein-DNA complexes were detected with these IAPP probes in these extracts (Fig. 6). Specificity of binding was determined in competition assays with wild-type IAPP and with wild-type and mutant alleles of the insulin −213/−192 element as competitors. The competition pattern indicates that there was at least one common, specific protein-IAPP element complex detected using βTC3 cell extract with the A1 and A2 probes (Fig. 6, A and B). This complex comprises the major β cell binding activity and co-migrates with the PDX-1-insulin complex (Fig. 6D); it was not detected in HeLa extracts (Fig. 6). The presence of PDX-1 in this complex was also confirmed by antibody-supershift analysis. Thus, the addition of an antibody that recognizes PDX-1 specifically super-shifted this complex with the IAPP A1 and A2 probes, whereas there was no effect upon incubation with a polyclonal antisera to the unrelated murine mesoderm-specific Mox-2 homeoprotein (Fig. 6). In contrast, the IAPP A3 element does not bind to this factor (Fig. 6C).

Our gel-shift results indicated that the homeoprotein PDX-1 was the principal A element binding protein of the IAPP gene. Recent studies by Bretherton-Watt et al. (19) have also come to a similar conclusion. However, Wang and Drucker (20) proposed that Isl-1, a LIM homeodomain protein, binds to and activates IAPP expression through an IAPP A/T-rich element. Their results indicate that Isl-1 actions are primarily through the A2 element. To compare the relative contribution of Isl-1 and PDX-1 with A2 element binding activity, we analyzed the...
effect of PDX-1 and Isl-1 polyclonal antisera on protein-DNA complex formation in extracts prepared from βTC-3 cells and human islets. Isl-1 antisera raised to either amino acids 86–175 or 178–349 were used in this analysis. Western blot analysis indicated that there was approximately 80-fold more PDX-1 than Isl-1 in βTC-3 cells and 1.5-fold more in islets (Fig. 7). The PDX-1 binding complex formed with the A2 element probe was quantitatively removed from these extracts upon addition of the PDX-1 antisera (Fig. 8). In contrast, the Isl-1 and preimmune antisera did not have any specific influence on A2 element complex levels. Increasing the amount of Isl-1 antisera had no effect on this result nor did changing the gel-shift reaction conditions to those of Wang and Drucker. These results strongly suggest that PDX-1 is the major IAPP A2 element binding factor in β cells, with little or no contribution from Isl-1. We infer from these results that PDX-1 is an activator of IAPP A/T-element driven activity in islet β cells.

**DISCUSSION**

IAPP is a major component of islet amyloid plaques found in NIDDM patients (1). Although the physiological and pathophysiological role of IAPP is unclear, this factor appears to inhibit insulin action by affecting both its release from the pancreas (5, 6) and its activity in target tissues such as skeletal muscle (7). It has been proposed that overexpression of IAPP could contribute to the development of disease (9). Recent studies with transgenic mice that overexpress human IAPP support this proposal (35). Thus, inhibiting IAPP transcription by targeting the action of factors uniquely required for expression could be a viable therapeutic initiative. In the present study, we have identified the cis-acting DNA elements of the human IAPP gene that are required for islet β cell expression. Our analyses revealed that human IAPP sequences, which reside between nucleotides 222 to 1450, contain the control elements that impart β cell-specific expression. Furthermore, we observed that transcription appears to be mediated through the interaction of both positive- and negative-acting cellular factors. These results also indicate that the PDX-1 transcription factor contributes to the correct, cell-type-specific expression of both the IAPP and insulin genes.

The transgenic reporter gene IAPP-GH, which contained human IAPP gene sequences from 2279 to 1450 bp driving growth hormone expression, was expressed in the same islet-specific manner as the endogenous gene and therefore served to define the region of the IAPP gene required for transcription. To determine more precisely the regions of the IAPP gene that
were important in β cell expression, we constructed a series of 5’- and 3’-flanking deletion mutants spanning the sequences from −2798 to +450 and examined their activity in IAPP expressing and nonexpressing cell lines. Two regions appear to be important in islet β-cells, a promoter proximal region between −222 and −91 bp and an intron region between +104 and +434 bp. The promoter region contains the cis-acting elements required for islet-specific transcriptional activity. In contrast, the intron region appears to be important in post-transcriptional control. This conclusion is based on our observation that replacement of IAPP first intron sequences with non-contrasting sequences from the ubiquitously expressed EF-1 gene did not affect the expression of other genes selectively transcribed in islet β-cells, like the glucose transporter 2, β glucokinasen, or glucagon-like peptide 1 receptor genes in their study. However, previous studies have demonstrated that the B-HLH-insulin transcripion may be distinguished by factors that bind to and activate expression at the A3 or E1 elements. Insulin transcription between insulin and IAPP may be distinguished by factors that bind to and activate expression at the A3 or E1 elements. Transcription between insulin and other mammalian genes (36, 37) and in plants (38) indicate that this may be an important regulatory process. Detailed experiments conducted by Huang and Gorman (39) suggest that splicing is coupled to efficient polyadenylation and transport of the mRNA to the cytoplasm.

To systematically identify the elements within the human IAPP proximal promoter region that were important in β cell expression, linker-scanner mutants were constructed spanning the sequences between −211 and −32. Our results suggest that activation of IAPP transcription is primarily mediated by an interplay between several distinct regulators acting at the A elements at −172/−163, −154/−142, and −91/−84 bp and the E-like site at −138/−131 bp. The activator of the A1 and A2 elements appears to be PDX-1, a factor which plays a key role in pancreatic determinisation and insulin transcription in β-cells (14–18). This conclusion is based upon several observations. First, antibodies to PDX-1 recognize the principal binding complex associated with each of these elements in gel shift experiments performed with β cell line and islet extracts. The recent binding and antibody supershift studies conducted with the human IAPP gene by Bretherton-Watt et al. (19) also support this conclusion. Second, the endogenous expression pattern of PDX-1 and IAPP appear to be identical (4, 12, 14, 16). Finally, Serup et al. (40) have recently demonstrated that PDX-1 stably transfected into the islet α cell line AN 697 coordinately induces IAPP and insulin gene expression. Interestingly, PDX-1 did not affect the expression of other genes selectively transcribed in islet β-cells, like the glucose transporter 2, β glucokinase, or glucagon-like peptide 1 receptor genes in their study. In contrast, co-transfection experiments conducted by Wang and Drucker (20) indicates that Isp-1 can activate A2 element-mediated expression. These studies were conducted in the islet α cell line, InR1 G9. Although we cannot detect any binding of Isp-1 to this element in either βTC-3 or islet extracts, it is still possible that there are circumstances where this generally distributed islet factor (41) is important for A2 element activity. However, we believe that the islet cell types used in the various investigations may explain the discrepancies between studies. Wang and Drucker (20) used an islet α cell line. In contrast, our results and those of Bretherton-Watt et al. (19) were obtained with IAPP producing β cell lines (10, 15, 21, 42). As a consequence, we believe that PDX-1 is the IAPP activator of A1- and A2-mediated expression in islet β and δ cells.

Given the ability of PDX-1 to induce both IAPP and insulin expression, there must be other regulators that control their distinct expression patterns. Transcription between insulin and IAPP may be distinguished by factors that bind to and activate expression at the A3 or E1 elements. Insulin transcription is also regulated by proteins in the B-HLH family (43). However, previous studies have demonstrated that the B-HLH-like site of the IAPP gene will not functionally substitute for the insulin B-HLH site (10), arguing that a distinct factor is important in control. Alternatively, the negative regulator that acts at the −111/−102-bp element or another of the cis-active

**Fig. 6.** Binding of βTC-3 and HeLa nuclear proteins to IAPP and insulin A element sequences. Equal concentrations of HeLa (lane 1) and βTC3 (lanes 2–7) protein extracts (5 μg) were analyzed for IAPP A1 (A), IAPP A2 (B), IAPP A3 (C), and insulin A2 element binding (D). Competition reactions were conducted with the A probe plus βTC-3 extract in the presence of a 100-fold molar excess of unlabeled competitor. Lane 3, corresponding IAPP A competitor; lane 4, wild-type insulin A2; and lane 5, mutant insulin A2. The αXHbox8 and αMox-2 antisera were preincubated with the βTC-3 extract before initiation of the DNA-binding reactions. Lane 6, plus αPDX-1 antibody; lane 7, αMox-2 antibody. The position of the PDX-1 complex is labeled.

**Fig. 7.** Western blot analysis for PDX-1 and Isl-1 proteins in βTC-3 and human islet extracts. The blot was probed with the polyclonal antibodies (1:500 dilution) raised to Isl-1 (K5) (A) and PDX-1 (B). The positions of PDX-1 and Isl-1 are indicated. The amount of βTC3 (lanes 1–4) and islet (lanes 5 and 6) extract protein in each lane varies. Lane 1, 5 μg; lane 2, 10 μg; lane 3, 15 μg; lane 4, 20 μg, lane 5, 5 μg; and lane 6, 10 μg. The exposure time for the βTC3 (lanes 1–4) in panel A is 2 min and in panel B is 10 s; the islet exposure time in panels A and B was 10 s. Quantitation of the relative levels of PDX-1 to Isl-1 was determined by densitometric scanning of the autoradiogram. There was approximately 80-fold more PDX-1 than Isl-1 in βTC3 extracts and about 1.5-fold more in islets.
elements identified by our linker-scanner analysis may be key to selective expression. Further understanding of the importance of each of these factors in IAPP transcription may lead to better therapeutic strategies for preserving β cell function in NIDDM.

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Note Added in Proof—During review of this manuscript, another report was published that describes the importance of PDX-1 in IAPP transcription in the β cell (Watada, H., Kajimoto, Y., Kaneto, H., Matsumaki, T., Fujitani, Y., Miyazaki, J., and Yamasaki, Y. (1996) FEBS Lett. 3821–3229).
