Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 9015–9020, August 1996
Developmental Biology

Induction of insulin and islet amyloid polypeptide production in pancreatic islet glucagonoma cells by insulin promoter factor 1

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Communicated by Donald F. Steiner, The University of Chicago, Chicago, IL, May 24, 1996 (received for review February 20, 1996)

ABSTRACT **Insulin promoter factor 1 (IPF1), a member of the homeodomain protein family, serves an early role in pancreas formation, as evidenced by the lack of pancreas formation in mice carrying a targeted disruption of the IPF1 gene [Jonsson, J., Carlsson, L., Edlund, T. & Edlund, H. (1994) *Nature (London)* 371, 606–609]. In adults, IPF1 expression is restricted to the β-cells in the islets of Langerhans. We report here that IPF1 induces expression of a subset of β-cell-specific genes (insulin and islet amyloid polypeptide) when ectopically expressed in clones of transformed pancreatic islet α-cells. In contrast, expression of IPF1 in rat embryo fibroblasts factor failed to induce insulin and islet amyloid polypeptide expression. This is most likely due to the lack of at least one other essential insulin gene transcription factor, the basic helix-loop-helix protein Beta2/NeuroD, which is expressed in both α- and β-cells. We conclude that IPF1 is a potent transcriptional activator of endogenous insulin genes in non-β islet cells, which suggests an important role of IPF1 in β-cell maturation.**

Insulin promoter factor 1 (IPF1) is expressed in precursor cells during pancreas ontogeny (1, 2), and expression is required for pancreas formation (3, 4). During ontogeny, IPF1 expression becomes restricted to the nuclei of the insulin-producing pancreatic islet β-cells, suggesting that maintenance of IPF1 expression is necessary for the differentiation islet β-cells from an IPF1-positive precursor common to all islet cells (2, 5). This restricted expression profile within the islets is reflected in the transplantable rat pancreatic insulinoma (IN) and glucagonoma (AN), which show substantial similarity to the mature islet β- and α-cells, respectively (6–8). Thus, the AN is lacking IPF1 expression, as is the normal α-cell, and was recently found to be similar to normal α-cells in its expression of glucokinase as well as of the glucose-regulated insulinotropic peptide and glucagon-like peptide 1 receptors (9, 10). *In vitro* IPF1 binds to multiple sites in the insulin promoter and activates insulin gene reporter constructs when cotransfected into cell lines (1, 5, 11, 12). This activity is dependent on cooperation between IPF1 and insulin enhancer factor-1 (IEF-1; refs. 5 and 12), a heterodimer composed of Beta2/NeuroD, which is present in both α- and β-cells, and ubiquitous class A helix-loop-helix proteins, such as Beta1/rat E-box binding protein (REB; ref. 13) and products of the E2A gene (E47, E12, and ITF-1; refs. 14–19). In addition to IPF1 and IEF-1 binding sites, transcriptional regulation of the insulin gene requires a number of other cis-elements to which factors not yet cloned are binding (20–23). To address whether IPF1 could activate transcription of the otherwise silent insulin genes in islet cells lacking IPF1 but expressing at least a subset of the other insulin gene transcription factors, a cDNA encoding rat IPF1 (24) under transcriptional control of the cytomegalovirus promoter was stably transfected into the glucagon-producing AN 697 rat islet α-cell line. As a control, we performed the IPF1 transfection in syngeneic New England Deaconess Hospital (NEDH) rat embryo fibroblasts (REFs), which lack most, if not all, of the factors interacting with the insulin gene regulatory elements.

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MATERIALS AND METHODS

Establishment of Tumors and Cell Lines. Tumors were allowed to form in NEDH rats by subcutaneous injection of 10^6 cells from each cell line as described (7). Circulating levels of glucagon and insulin were measured by radioimmunoassays as described (8). Blood glucose was measured as described (7). Animals were handled according to permission obtained from the Committee for Inspection of Animal Experiments under the Danish Ministry of Justice. The AN 697 cell line was established from the AN, MSL-G-AN (6), using procedures as described (25) and cultured in RPMI 1640 medium (GIBCO/BRL) supplemented with 10% fetal calf serum, 10% NHI-6F-INS-conditioned medium, 2 mM L-glutamine, penicillin (100 units/ml), and streptomycin (100 μg/ml; GIBCO/BRL). NHI-6F-INS cells were grown as described (7). NEDH REFs were prepared using a modified 3T3 protocol (26) as follows. Rat embryos were dissected at embryonic day 14, minced, briefly treated with 0.25% trypsin, vortexed, and washed in RPMI 1640 medium with 10% fetal calf serum. Large aggregates were removed by sedimentation. Fibroblasts were cultured to homogeneity over >25 weekly passages. Growth crisis occurred at passage 16–17, after which surviving cells were propagated further in DMEM (GIBCO/BRL) supplemented with 10% fetal calf serum, 2 mM L-glutamine, penicillin (100 units/ml), and streptomycin (100 μg/ml; GIBCO/BRL).

Transfection and Generation of Stable Lines. Plasmids encoding rat IPF1 (pCMV-STF-1; ref. 24) and neomycin resistance (pJNL-1; ref. 27) were introduced into AN 697 and NEDH REF cells by electroporation as described (28), followed by selection using G418 at 125 μg/ml for AN 697 cells and 2 mg/ml for NEDH REF cells.

Nuclear Extract Preparation, Electrophoretic Mobility-Shift Assay (EMSA), and Antibodies. Nuclear extracts were prepared as described (29). EMSA analysis for detection of E-box binding factors were performed as described (30) using 5 μg of the indicated nuclear extracts. Competition was performed by adding 200-fold molar excess of the indicated

Abbreviations: IPF1, insulin promoter factor 1; IN, transplantable rat pancreatic insulinoma; AN, transplantable rat pancreatic glucagonoma; IEF-1, insulin enhancer factor-1; REF, rat embryo fibroblast; EMSA, electrophoretic mobility-shift assay; RT-PCR, reverse transcriptase-PCR; IAPP, islet amyloid polypeptide; GLP-1-R, glucagon-like peptide 1 receptor; GLUT2, glucose transporter-2; NEDH, New England Deaconess Hospital; REB, rat E-box binding protein.

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oligonucleotides before incubation with the labeled probe. Antibody supershifts were performed by addition of 1 μl of the antiserum before incubation with the labeled probe. EMSA analysis for detection of IPF1 was performed similarly, except that electrophoresis of native polyacrylamide gels were performed in 0.5× TBE (90 mM Tris/64.6 mM boric acid/2.5 mM EDTA, pH 8.3). Polyclonal anti-IPF1 antiserum (Ab 1859) was raised against a glutathione *S*-transferase–IPF1 fusion protein containing the 63 C-terminal amino acids of rat IPF1 as described (12). Yae mAb And rabbit polyclonal antibody against human E-box binding protein (crossreacts to REB) was from Santa Cruz Biotechnology. Insulin mAb HUI18 was from Novo Biolabs (Bagsværd, Denmark; ref. 25).

Immunoblot and Reverse Transcriptase-PCR (RT-PCR) Analysis. Immunoblot was performed as described (31) using 15 μg of nuclear extract resolved by SDS/PAGE. Anti-IPF1 antiserum 1859 was used as primary antibody followed by visualization with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody.

RT-PCR was performed on total RNA prepared by the RNAzol method (Cinna/Biotecx Laboratories, Friendswood, TX). cDNA synthesis was performed as follows: total RNA was diluted in diethyl pyrocarbonate treated water to 0.2 mg/ml, denatured at 85°C for 3 min, and quickly chilled on ice. Five microliters of the total RNA was mixed with 20 μl of RT-mix [50 mM Tris·HCl, pH 8.3/75 mM KCl/3 mM $MgCl_2$/10 mM DTT/200 units of Moloney murine leukemia virus reverse transcriptase (GIBCO/BRL)/40 units of RNAsin (Promega)/3 μg of random hexamers (GIBCO/BRL)/0.9 mM of dNTPs (Pharmacia); all in final concentrations] and left 10 min at room temperature and subsequently incubated at 37°C for one hr. After cDNA synthesis, the reaction was diluted with 50 μl of H_2O. Fifty-microliter PCRs contained 3 μl of the diluted cDNA and 47 μl of PCR mix {50 mM KCl/10 mM Tris·HCl, pH 9.0 at 25°C/0.1% Triton X-100/1.5 mM $MgCl_2$/40 mM dATP, dTTP, and dGTP/20 mM dCTP/10 pmol of each primer/2.5 units of *Taq* polymerase (Promega)/2.5 μCi of [α-^{32}P]-dCTP (3000 Ci/mmol; Amersham)}. Primers for the first reaction were as follows: for neomycin, sense, 5′-GTCTTGTCGATCAGGATGATCTG-3′, and antisense, 5′-CAATATCACGGGTAGCCAACGC-3′; for TATA-box binding protein, sense, 5′-ACCCTTCACCAATGACTCCTATG-3′, and antisense, 5′-ATGATGACTGCAGCAAATCGC-3′; and for plasmid-derived IPF1 mRNA, sense, 5′-TAATACGACTCACTATAGGGAGA-3′, and antisense, 5′-CCACCCCAGATCGCTTTGACA-3′. All these primers were used in one reaction (denaturing at 94°C for 1 min followed by 25 cycles at 94°C for 30 sec/55°C for 1 min/72°C for 1 min). Primers for a second reaction were as follows: for insulin, sense, 5′-TCCTGCCCCTGCTGGCCCTGC-3′, and antisense, 5′-AGTTGCAGTAGTTCTCCAG-3′; for α-tubulin, sense, 5′-GCGTGAGTGTATCTCCATCCA-3′, and antisense, 5′-GGTAGGTGCCAGTGCGAACTT-3′; and for islet amyloid polypeptide (IAPP), sense, 5′-AGTCCTCCCACCAACCAATGT-3′, and antisense, 5′-AGCACAGGCACGTTGTTGTAC-3′. The second reaction consisted of 18 cycles with the thermal profile as in the first reaction. Primers for a third reaction were as follows: for glucose transporter-2 (GLUT2), sense, 5′-GACACCCCACTCATAGTCACA-3′, and antisense, 5′-CAGCAATGATGAGAGCATGTG-3′; for glucagon-like peptide 1 receptor (GLP-1-R, sense, 5′-GTTCACAGAGCTCTCCTTCACT-3′, and antisense, 5′-AATTTTGGCAGGTGGCTGCATACA-3′); for glucokinase, sense, 5′-GTGATGTGGTCTGTGGCCAA-3′, and antisense, 5′-TCTGCTCCATACTAGCTCTG-3′; and for glucose-6-phosphate dehydrogenase, sense, 5′-GACCTGCAGAGCTCCAATCAAC-3′, and antisense, 5′-CACGACCCTCAGTACCAAAGGG-3′. The third reaction consisted of 25 cycles with the thermal profile as in the first reaction. Reaction products were separated on 0.4-mm 7 M urea/1× TBE/6% polyacrylamide gels. The gels were dried and autoradiographed. The number of cycles chosen for each particular reaction ensures that amplification stops in the exponential phase, allowing semiquantitative measurements to be obtained on a Molecular Dynamics PhosphorImager series 400 with band intensities calculated using IMAGEQUANT software (32).

RESULTS

AN 697 but not NEDH REF Cells Contain IEF-1. As IPF1 relies on cooperation with IEF-1 in transient transfection assays, we first determined that AN 697 cells but not NEDH REF cells contained IEF-1. Using the EMSA with a probe containing the proximal E-box (E1; Fig. 1*A*) from the rat insulin 1, we found one complex specific for the E-box, as judged by the ability of excess of cold oligonucleotide to compete for binding in contrast to an oligonucleotide mutated in the E-box (E1M1; Fig. 1*A*), which could not compete (Fig. 1*B*, compare lanes 10–12 with lanes 13–15). This complex was present in the insulin producing NHI-6F-INS and in AN 697 but not in NEDH REF cells (Fig. 1*B*, lanes 1–3). The complex was identified as IEF-1 by the reactivity with the mAb Yae, which is specific for E2A gene products (33–35) but not a control mAb OX-18 (Fig. 1*B*, compare lanes 4–6 with lanes 7–9). The complete supershift observed with the Yae mAb suggests that the IEF-1 complex we observe in AN 697 and NHI-6F-INS cells does not contain Beta1/REB, the other class A member of the helix-loop-helix family that can form

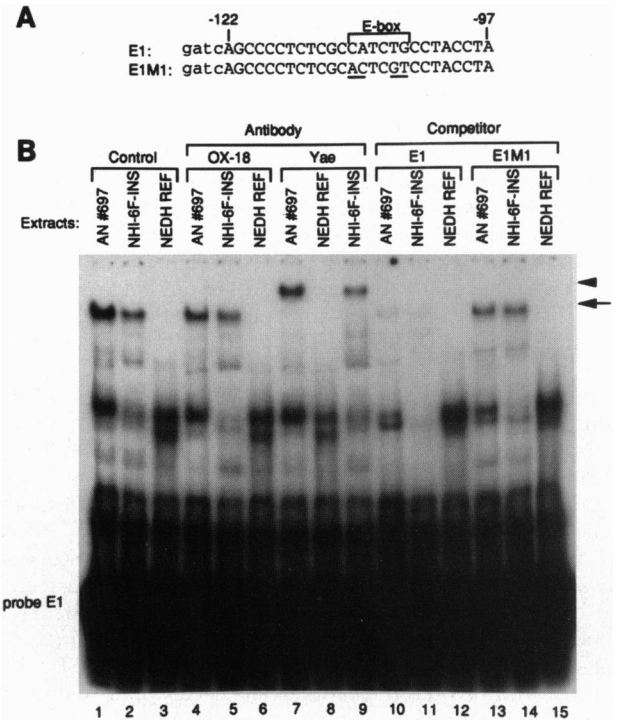

FIG. 1. Helix-loop-helix factor IEF-1 is present before expression of IPF1. (*A*) Sequence of oligonucleotides used in the EMSA (sense strand only) representing the E1-box of the rat insulin I gene. Wild-type (E1) and mutant (E1M1, mutated nucleotides underlined) forms of the rat insulin 1 gene proximal IEF-1 binding site and the position relative to the transcriptional start site are shown. Lowercase letters indicate nucleotides present only for cloning purposes. (*B*) EMSA analysis of E-box binding proteins. One E-box-specific complex (competed with excess of unlabeled E1 oligonucleotide but not with excess E1M1) was selectively present in the islet cells (arrow, compare lanes 10–12 with lanes 13–15). The complex was identified as IEF-1 by the reactivity with mAb Yae specific for E2A gene products but not the control mAb OX-18 (arrowhead, compare lanes 7–9 with lanes 4–6).

part of IEF-1. In accordance with this, we did not observe any reactivity of the IEF-1 complex with an antiserum that recognizes REB (data not shown).

Expression of IPF1 in AN 697 Cells Reestablish the Full Complement of A4/A3 Binding Factors. Plasmids encoding rat IPF1 and neomycin resistance were introduced into AN 697 and NEDH REF cells by electroporation, and stable clones were selected in G418. Twelve G418-resistant colonies of AN 697 cells were picked, expanded, and screened by immunocytochemistry for expression of the introduced IPF-1 cDNA. Four cultures expressed IPF1 protein homogenously, whereas three cultures did not express detectable IPF1. The homogeneity of these cultures suggests that they represent true clones. Five cultures expressed IPF1 in a subset of the cells, these latter cultures probably representing mixture of IPF1-expressing and nonexpressing clones.

IPF1 binds two A+T-rich elements (A1 and A4/A3; ref. 20) in the rat insulin 1 gene (5, 12, 36). Additionally, the A4/A3 site binds other factors, one of which is expressed in a β-cell-specific fashion (37, 38). Performing EMSA using a probe spanning the A4/A3 elements (Fig. 2*A*), we analyzed for the presence of these factors in AN 697 cells and in four transfected clones, including two positive and two negative for IPF1. In addition to detecting IPF1 (identified with an anti-IPF1 rabbit polyclonal antibody) in the two positive transfectants as expected (Fig. 2*B*, compare lanes 20 and 21 with 26 and 27) the A4/A3 probe bound two sequence specific low mobility complexes (Fig. 2*B*, compare lanes 7–12 with lanes 13–18), both of which were expressed at comparable levels regardless of IPF1 expression (Fig. 2*B*, lanes 1–6). These complexes were also seen using extract from the NHI-6F-INS cell line. We cannot unambigously determine whether one of these complexes represent the previously identified β-cell-specific low mobility complex IEF-2 (37), but neither of these complexes arise when using nuclear extract from NEDH REF cells (data not shown). The analysis thus revealed that the introduction of IPF1 expression in AN 697 cells reestablished the full complement of A4/A3 binding proteins as found in NHI-6F-INS (Fig. 2*B*, lanes 24 and 30). Furthermore, the

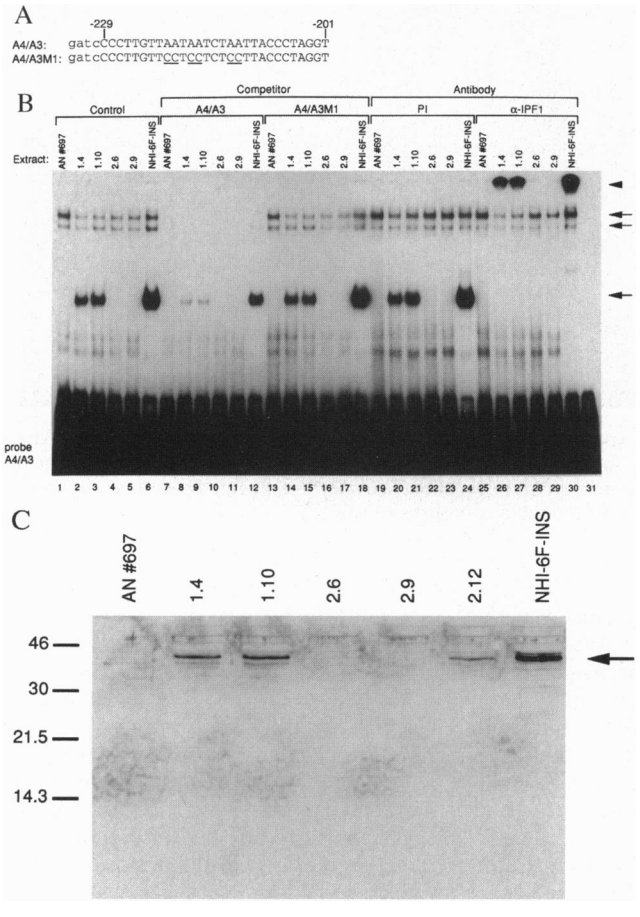

FIG. 2. IPF1 is the only A4/A3 element binding protein missing from AN 697 cells. (*A*) Sequence of the A4/A3 oligonucleotides used in the EMSA (sense strand only). Wild-type (A4/A3) and mutant (A4/A3M1, mutated nucleotides underlined) forms of the rat insulin 1 gene distal IPF1 binding site and the position relative to the transcriptional start site are shown. Lowercase letters indicate nucleotides present only for cloning purposes. (*B*) EMSA analysis of A4/A3 element binding proteins in nuclear extracts from the indicated cell lines. Low mobility TAAT motif-specific complexes are indicated with shaded arrows. IPF1 binding is indicated by the arrow and is identified by antibody supershift with anti-IPF1 antiserum (α-IPF1; arrowhead) but not with preimmune serum (PI). These complexes are also seen using extracts from NHI-6F-INS. (*C*) Immunoblot analysis for IPF1 production performed using equivalent amounts of nuclear extracts from the indicated cell lines. Molecular sizes are indicated in kDa.

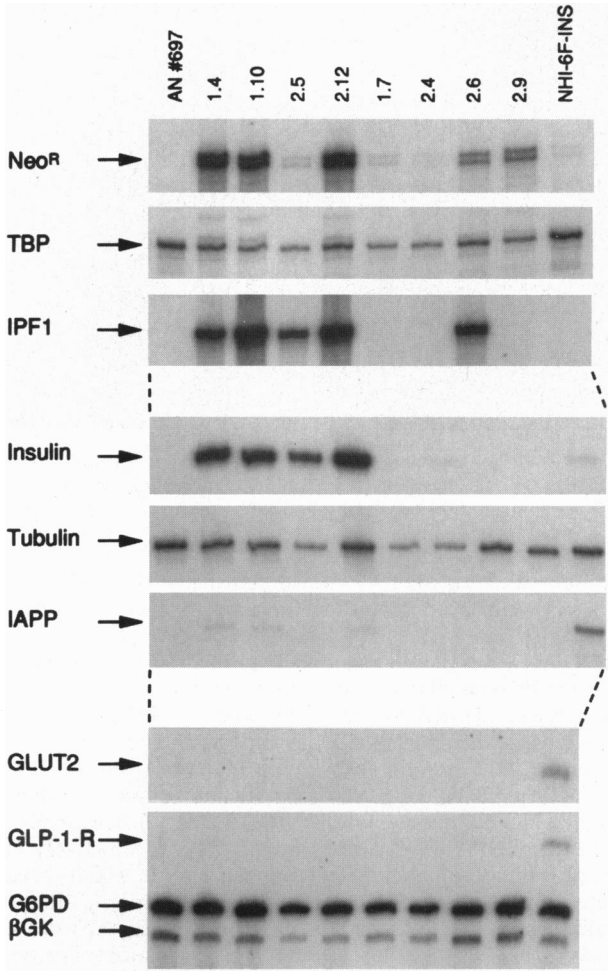

FIG. 3. mRNA expression of insulin and IAPP follows transfected IPF1. Seven homogeneous clonal cultures [four IPF1-positive (clones 1.4, 1.10, 2.5, and 2.12), three IPF1-negative (clones 1.7, 2.6, and 2.9), and one heterogeneous culture (clone 2.4)] were subjected to RT-PCR analysis. RT-PCR analysis of IPF1, neomycin, insulin, and IAPP mRNA expression on total RNA from the indicated cell lines. TATA-box binding protein (TBP) and tubulin were used as internal controls. Of the five clones expressing message from the introduced IPF1 cDNA, four have begun expressing insulin and IAPP, with clone 2.6 as the exception. The neomycin/human insulin gene transfected NHI-6F-INS cell line is acting as positive control. The absence of IPF1 message in NHI-6F-INS is due to the sense IPF1 primer, which anneals to a sequence present only in plasmid-derived IPF1 mRNA.

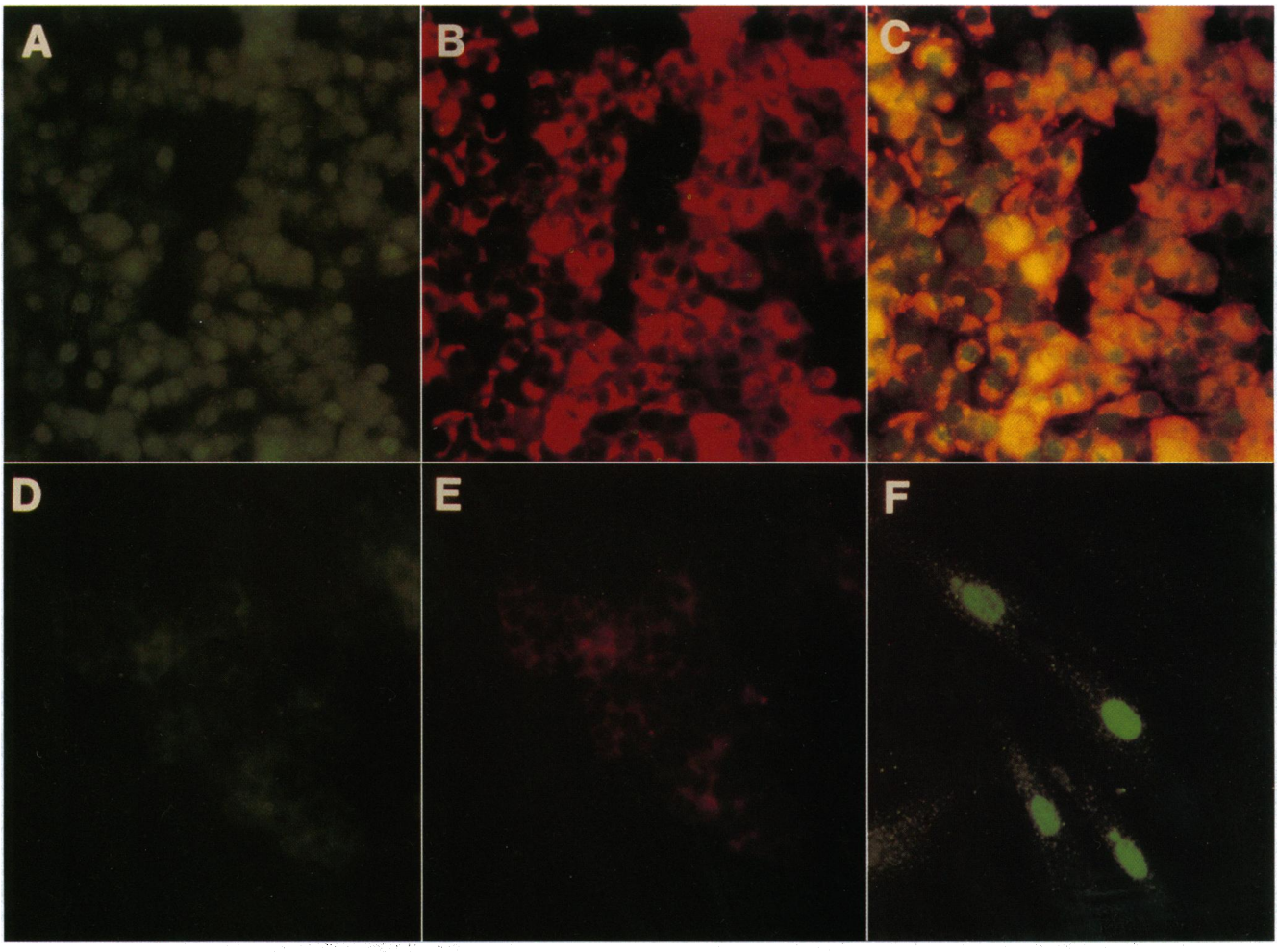

FIG. 4. IPF1-expressing NEDH REF cells do not produce insulin. Cells [AN 1.10 (*A–C*), AN 2.6 (*D* and *E*), and a pool of IPF1-transfected NEDH REF cells (*F*)] were grown in chamber slides and stained with antiserum against IPF1, followed by fluorescein isothiocyanate (FITC)-conjugated secondary antibody. The cells were also stained with a mAb to insulin followed by Texas Red-conjugated secondary antibody. FITC exposures (*A* and *D*), Texas Red exposures (*B* and *E*), FITC, and Texas Red double exposures (*C* and *F*).

introduced IPF1 protein was indistinguishable from the native form in insulin producing β-cell cultures by Western blotting (Fig. 2*C*).

IPF1 Activates the Endogenous Insulin and IAPP Genes. To assay for IPF1-mediated changes in phenotype, the expression levels of several mRNAs were analyzed by RT-PCR. All clones expressed neomycin message as expected. The four clones expressing IPF1 protein also showed message from the introduced IPF1 cDNA as expected. Significantly, these four clones had all begun expressing insulin mRNA (Fig. 3). The levels of insulin mRNA were determined semiquantitatively by PhosphorImager analysis in relation to tubulin, which served as an internal standard. The insulin/tubulin ratio of the four insulin-producing clones varied between 2.0 (clone 2.5) and 6.4 (clone 2.12). These levels were comparable to the *in vivo* grown IN, which had a insulin/tubulin ratio of 13.1. This should be compared with newborn rat islets, where the insulin/tubulin ratio is ≈100 (32). Significantly, the IPF1-transfected, insulin-producing AN clones show ≈10-fold higher insulin mRNA levels than the *in vitro* grown rat insulinoma cell line RIN 5AH (32). Importantly, expression of the IAPP gene, which shares certain transcription factors with the insulin gene (39) including IPF1, was detected in the clones that had activated the insulin gene (Fig. 3). The IAPP/tubulin ration varied between 0.1 (clone 2.5) and 0.2 (clones 1.4, 1.10, and 2.12), which is ≈5-fold lower than in the *in vivo* grown IN and 50-fold lower than in newborn rat islets (32). One clone (clone 2.6) was scored negative for IPF1 by immunocytochemistry but did express at least the part of the introduced IPF1 message detectable by the primers used in the RT-PCR. However, in agreement with the immunocytochemical data (Fig. 4 *D* and *E*), Western blotting revealed that no IPF1 protein was produced (Fig. 2*C*), while it was readily detected in clones 1.4, 1.10, and 2.12 and in the IN-derived NHI-6F-INS cell line. A weak insulin mRNA signal observed in culture 2.4 is likely explained by the heterogeneity of the culture with only few cells expressing IPF1. Immunocytochemistry revealed that culture 2.4 indeed contained a small fraction of insulin-producing cells. This was contrasted by the observation that clones 1.4, 1.10, 2.5, and 2.12 produced insulin in close to 100% of the cells (shown for clone 1.10 in Fig. 4 *A–C*). As in the mouse αTC1.9 cells (5), transient expression of IPF1 in AN 697 cells did not induce insulin production as assayed by immunocytochemistry (data not shown). IPF1 expressed in NEDH fibroblasts was clearly detectable by immunocytochemistry and characterized by a strict nuclear localization of immunoreactive IPF1 (Fig. 4*F*); however, in contrast to the situation in AN 697 cells, IPF1 expression induced neither insulin (Fig. 4*F*) nor IAPP expression (data not shown).

IPF1-Expressing AN 697 Cells Acquire a Hypoglycemic Phenotype *in Vivo* but Retains the AN-Associated Anorexia. *In vivo* passage of IPF1-expressing AN 697 cells further emphasized the acquired ability of these cells to produce insulin, as the animals receiving clones 1.4, 1.10, and 2.12 all developed severe hypoglycemia (Table 1) and a degree of hyperinsulin-

Table 1. Circulating levels of insulin, glucagon, and blood glucose values in animals carrying tumors induced by injection of the indicated cell lines

Animal no.	Cell line	IPF1	Insulin, pM	Glucagon, pM	Blood glucose, mM
188	AN 697	−	225	2894	4.3
189	AN 697	−	53	1451	4.6
190	1.4	+	6462	718	3.3
191	1.4	+	10393	729	0.1
193	1.10	+	4612	426	0.6
193	1.10	+	—*	—*	1.5
194	2.6	−	24	1133	4.5
195	2.6	−	30	909	5.3
196	2.9	−	18	816	3.5
197	2.9	−	272	1609	4.3
198	2.12	+	10597	1252	0.3
199	2.12	+	7153	1171	1.9
Normal range†			470–900	20–60	4.0–5.0

*Died before serum samples could be obtained.
†See ref. 8.

emia (up to 10 nM), which is unmatched even by the genuine IN (7, 8).

Importantly, the potential of AN 697 cells to induce anorexia was not affected by expression of IPF1, showing that a complete shift of the phenotype was not achieved, but rather that the AN cells simply gained the capacity to also produce the two β-cell hormones. Hyperglucagonemia, which is a characteristic of animals carrying tumors induced by the parental AN 697 cells (6, 8), was not affected by IPF1 expression in all animals tested (Table 1). In addition, IPF1-negative clones (clones 2.6 and 2.9) have maintained the ability of the parental AN to induce anorexia and hypoinsulinemia (8).

GLUT2, GLP-1-R, and Glucokinase Expression Is Not Affected by IPF1. Three additional genes (GLUT2, glucokinase, and GLP-1-R), which are normally expressed in the β-cells within the pancreatic islets, were examined. GLUT2 mRNA could not be detected, while GLP-1-R mRNA was detected at very low levels in all G418-resistant clones regardless of IPF1 expression, whereas both were highly expressed in the IN-derived NHI-6F-INS (Fig. 3). The glucokinase gene, which contains three consensus IPF1 binding sites (40), was transcriptionally active before introduction of IPF1, and expression levels were not altered in any of the eight G418-resistant clones (Fig. 3). IPF1 has been implicated in somatostatin gene activation (24, 36), but we found that none of the IPF1-positive clones contained detectable somatostatin mRNA (data not shown).

DISCUSSION

Taken together, our results suggest that activation of the hormone genes coding for insulin and IAPP is controlled by IPF1. This control must, however, rely on cooperation with other factors as IPF1 is not capable of insulin gene activation in NEDH REF cells. Other genes showing β-cell restriction within the islet, such as the genes for GLUT2, glucokinase, and the GLP-1-R are apparently outside the immediate control of IPF1. The lack of effect by IPF1 upon expression of these genes as well as on the AN phenotype shown by AN 697 cells grown *in vivo* indicates that other factors necessary for a complete β-cell phenotype are yet to be defined. The successful activation of the insulin gene in AN 697 cells but not in NEDH fibroblasts are probably due to selective presence of the helix-loop-helix factor IEF-1 in AN 697 cells, as it is in other α-cell lines (16, 18). A number of studies have shown that the insulin gene cis-elements to which IEF-1 binds (the E-boxes) are those most sensitive to mutational inactivation (21, 23, 41, 42). IEF-1 can apparently be divided into several distinct complexes with a common cell-specific component (Beta2/NeuroD) forming heterodimers with at least two different class A members of the helix-loop-helix family (13). We did, however, selectively detect complexes containing E2A gene products but not REB in our cell lines. Furthermore, the importance of the E2A-containing complexes was recently shown through inhibition of E2A expression by E2A antisense RNA, which abolished insulin gene transcription in RIN insulinoma cells (43). However, the effect on REB expression was not addressed in that study, which raises the possibility that REB expression was also inhibited by the E2A antisense RNA due to the high homology between these two genes. In addition to IEF-1, it is most likely that other islet-specific factors necessary for insulin expression such as the RIPE3b1 binding factor (22, 23) are selectively present in AN 697 cells when compared with NEDH REF cells and thus rendering the former permissive for insulin gene activation.

As we have previously found for αTC1.9 cells transient expression of IPF1 in AN 697 cells failed to induce insulin gene activation. The reason for this remains speculative, but it might indicate that IPF1 induces expression of an unknown factor required for insulin gene activity and that the time period from transfection to immunocytochemical analysis (40 hr) is too short to allow sufficient amounts of this putative factor to be synthesized. Additionally, although we often find IPF1 expressed in pairs of neighboring cells, indicating that a single transfected cell has undergone cell division, we cannot formally exclude the possibility that the IPF1-expressing cells detected after 40 hr have yet to undergo a necessary mitosis.

The ability to induce expression of two major β-cell products by introducing a single transcription factor into an islet α-cell line suggests that heterologous cells can be induced to differentiate into β-cells *in vitro* by introduction of key regulatory factors. The present assay for IPF1 activity opens an unique opportunity to determine functional domains in the IPF1 protein.

We would like to thank Ragna Jørgensen, Heidi I. Jensen, Erna E. Pedersen, and Tove Funder for expert technical assistance. This work was supported by The Danish National Research Foundation [P.S., J.J., and F.G.A. (Center for Gene Regulation and Plasticity in the Neuroendocrine Network)]. The Hagedorn Research Institute is an independent basic research component of Novo Nordisk.

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