PDX-1 (also known as IDX-1/STF-1/IPF-1) is a homeodomain transcription factor evolutionarily derived from the Parahox amphioxus three-gene cluster and is a critical determinant of pancreas development in higher vertebrates (1–4). The early embryonic expression of PDX-1 demarcates the dorsal and ventral regions of the primitive mouse gut from which the two pancreatic buds emerge. As development proceeds, PDX-1 is expressed in the pancreas, the posterior portion of the developing primitive stomach epithelium, the anterior region of the duodenum, and the yolk sac (5). In the fully developed adult pancreas, PDX-1 expression is restricted predominantly to the endocrine pancreas (islets of Langerhans) in the insulin-producing β-cells and in a fraction of the somatostatin-producing δ-cells, as well as in a subpopulation of cells in the pancreatic ducts that are pluripotential progenitors for β-cells (6,7). PDX-1 is a transcriptional activator of the insulin, somatostatin, glucokinase, and GLUT2 genes (2–4), and is involved in regulating glucose responsiveness of the insulin promoter (8,9).

The importance of PDX-1 in pancreatic development is demonstrated by the consequences of the loss of PDX-1 function. Targeted disruption of the pdx-1 gene in mice and an inactivating mutation of pdx-1 (ipf-1) in a human infant (10) manifest as agenesis of the pancreas (11,12). Conditional disruption of the pdx-1 gene selectively in β-cells of mice using the Cre/Lox approach results in a progressive loss of β-cells and the development of diabetes in mice by age 5–6 months (13). Mice (14) and humans (10) with a haploinsufficiency for PDX-1 (IPF-1) develop diabetes and/or glucose intolerance.

The endocrine and exocrine compartments of the mature pancreas originate from a common undifferentiated pluripotential epithelium within the primitive gut tube at embryonic day (e) 9 of mouse development (15). These early PDX-1–expressing precursors of the pancreas differentiate into the branching ducts and a surrounding mass of epithelial cells. Between e14 and e16, differentiation of the epithelial cell mass into exocrine and endocrine lineages begins. By e16.5, PDX-1 expression in the exocrine lineage diminishes, and at e19, it is mostly restricted to the endocrine islets (16). PDX-1 gene expression in the exocrine pancreas is observed at a low level in a nonuniform lobular pattern in young and adult PDX-1–LacZ–expressing animals (17).

The consequences of a gain in function of PDX-1 during pancreas development have not been examined. In earlier studies, we misdirected the expression of PDX-1 to gut mesoderm using the HOXa4 promoter and observed a homeotic phenotype of cecal agenesis (18). However, ectopic expression of known PDX-1 target genes was not observed. This could be because certain required additional pancreatic factors are absent in mesodermal cells.
This result led to the hypothesis that ectopic expression of PDX-1 in a cell type more closely related to the endocrine pancreatic developmental lineage would allow PDX-1 to direct an endocrine program of differentiation. Therefore, we sought to determine whether targeted overexpression of PDX-1 in the exocrine compartment of the developing pancreas would allow for respecification of the exocrine cells.

To test this hypothesis, we directed overexpression of PDX-1 in acinar cells using the rat elastase-1 promoter. The elastase-1 gene is first expressed at e14–e15, specifically in the exocrine pancreas (19). Thus the elastase promoter is ideal for targeting expression of PDX-1 to the developing pancreatic epithelium that is destined to become the exocrine pancreas at e16–e17, when the expression of the endogenous pdx-1 gene is downregulated in the acinar cell lineage. By maintaining expression of PDX-1 in the exocrine pancreas lineage via transgenic expression of the elastase promoter–driven PDX-1, we postulated that development of the undifferentiated pancreatic epithelium
TABLE 1 β-cell mass measurements in control and TG mice

<table>
<thead>
<tr>
<th></th>
<th>Total β-cell mass/volume</th>
<th>Total β-cell number (× 10⁶)</th>
<th>Mean β-cell volume (µm³)</th>
<th>Total pancreas mass/volume</th>
<th>Volume-fraction of β-cells in pancreas (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 5 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDX-1 TG (n = 8)</td>
<td>0.14 ± 0.02 mm³</td>
<td>ND</td>
<td>ND</td>
<td>3.06 ± 0.55 mm³</td>
<td>4.7 ± 0.8†</td>
</tr>
<tr>
<td>Control (n = 8)</td>
<td>0.15 ± 0.04 mm³</td>
<td>ND</td>
<td>ND</td>
<td>4.91 ± 1.01 mm³</td>
<td>3.2 ± 1.0†</td>
</tr>
<tr>
<td>Age 6 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDX-1 TG (n = 6)</td>
<td>0.89 ± 0.14 mm³ᵇ</td>
<td>6.2 ± 1.0ⁿ</td>
<td>1408 ± 37ⁿ</td>
<td>46.7 ± 5.5 mm³</td>
<td>1.9 ± 0.17‡</td>
</tr>
<tr>
<td>Control (n = 8)</td>
<td>0.93 ± 0.12 mm³</td>
<td>6.9 ± 0.8</td>
<td>1341 ± 47</td>
<td>170.0 ± 11 mm³</td>
<td>0.54 ± 0.04</td>
</tr>
<tr>
<td>Age 8 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDX-1 TG (n = 6)</td>
<td>1.91 ± 0.26 mgⁿ</td>
<td>ND</td>
<td>ND</td>
<td>86 ± 8 mg</td>
<td>2.19 ± 0.18†</td>
</tr>
<tr>
<td>Control (n = 6)</td>
<td>1.66 ± 0.18 mg</td>
<td>ND</td>
<td>ND</td>
<td>247 ± 10 mg</td>
<td>0.67 ± 0.05</td>
</tr>
</tbody>
</table>

Data are means ± SE. *NS; †P < 0.005; ‡P < 0.0001; ND, not determined.

would be shunted from the exocrine into the endocrine pancreas. Surprisingly, we observed a marked dysmorphogenesis of the exocrine pancreas. We did not observe an increase in islet mass, but did find an enhancement of insulin secretion, suggesting that PDX overexpression in the exocrine pancreas is able to influence β-cell function.

RESEARCH DESIGN AND METHODS

Creation of transgenic mice. A 0.5-kb fragment of the rat elastase-1 promoter (19) was ligated into the plasmid PDX-1 (R1m) RGBpBS-KS containing the entire coding sequence of rat PDX-1. The strategy of the vector construction has been described in detail elsewhere (18). The recombinant plasmid also contains the 0.64-kb rabbit β-globin splicing signal cassette between the PDX-1 cDNA and the elastase promoter and the 0.52-kb rabbit β-globin polyadenylation signal cassette 3′ to the PDX-1 cDNA (20). DNA sequencing and polymerase chain reaction (PCR) analyses confirmed the proper orientation of the PDX-1 cDNA.

Transgenic (TG) mice were produced at the Beth Israel Transgenic Mouse Facility (Boston, MA) by standard transgenic protocols. DNA was isolated from tail snips obtained from mice aged 4 weeks. Tails were digested with proteinase K (Boehringer Mannheim, Indianapolis, IN) and the DNA was precipitated with ethanol. Genomic DNA was analyzed by PCR using primers chosen so that the total and mean β-cell volume could be calculated. The total pancreatic volume was likewise estimated by the fractionator principle, except that the final magnification was 84×.

From mice aged 8 months, the pancreases were removed and weighed before fixation and paraffin embedding. A set of sections (n = 6–9) was sampled by SURS from each pancreas. Unbiased estimates of volume fractions of β-cells, pancreas, and fat tissue were determined by the Delesse principle (24) using an Olympus BH-2 microscope equipped with a video camera and an automated x-y stepper and connected to a computer with the C.A.S.T-grid software (Olympus). Sampling fractions were chosen so that ~100 cells were counted per pancreas. Because all sampling fractions were known, unbiased estimates of the total number of β-cells and the total and mean β-cell volume could be calculated. The total pancreatic volume was likewise estimated by the fractionator principle, except that the final magnification was 84×.

Intraperitoneal glucose tolerance test. Age-matched female FVB control or TG mice, aged 6 weeks, were housed on a 12-h light/dark cycle. Food was removed at 2:30 p.m. before the glucose challenge. At 9:00 a.m., mice were administered intraperitoneally 1.5 g of glucose per kilogram of body weight. Blood was withdrawn at 0, 1, 15, 30, 60, and 120 min by orbital eye bleeding, and blood glucose was measured using a Hypocount MX B glucose monitor (Hermedico A/S, Copenhagen, Denmark). Insulin. For six control and six TG mice, the pancreases were weighed on a Mettler analytical balance and immediately frozen on dry ice. The tissue was lyophilized, extracted in 3 mol/l acetic acid three times, lyophilized again, and reconstituted in 0.1 mol/l acetic acid. Protein was measured using the Bradford Assay (Biorad), and insulin was measured with an enzyme-linked immunosorbent assay.

Multiplex reverse transcription-polymerase chain reaction. The multiplex reverse transcriptase (RT)–polymerase chain reaction (PCR) method described previously in detail (25) allows the coamplification of several cDNA products in a single tube. Briefly, RNA was prepared from pancreases using RNAzol (Cinna Biotex), according to manufacturer's instructions. cDNA was synthesized from 0.2 µg/µl of RNA, and all samples were diluted to the same final cDNA concentration. PCR was performed for 18–28 cycles, depending on the primer set. Internal standards
Enhanced expression of transgenes exclusively in the exocrine pancreas of normal FVB mice, these cells are not recognized by mouse PDX-1 (Fig. 1B). Transgenic PDX-1 expression was maintained at all ages examined (up to age 8 months).

Alteration in pancreas size. The size of the pancreases in the founder mice increased markedly as the mice aged. Five of the original founder mice were killed around age 1 year, and their body and pancreas weights were determined. The pancreases of the founder mice averaged 550 ± 45 mg wet wt, whereas the pancreases of the control animals of the same age weighed significantly less at 265 ± 35 mg (P < 0.002). Although there was a trend toward a larger body weight (data not shown), the differences were not significant (P < 0.10). No differences were found between TG mice and control non-TG littermates in liver, spleen, or kidney weight at any of the ages examined (data not shown). Analysis of the organ weights in the F1 generation showed a trend toward a smaller pancreatic weight, with no differences detected for the other organs. The homozygous El-16 animals age 8 months had a significantly smaller pancreas compared with age-matched control animals (158 ± 10 vs. 259 ± 11 mg wet wt, respectively; n = 6; P < 0.001).

Enhanced β-cell neogenesis but not β-cell mass. Although single insulin-positive cells can be found in the exocrine pancreas of normal FVB mice, these cells are found in greater numbers in the exocrine pancreas of TG

RESULTS

Transgene expression. To examine the effect of mis-directed overexpression of PDX-1 on the exocrine pancreas, 0.5 kb of the 5′ flanking region of the rat elastase-1 gene containing the promoter was fused to the coding sequences of the rat PDX-1 gene (20). The elastase 0.5-kb promoter fragment has previously been shown to drive the expression of transgenes exclusively in the exocrine pancreas (19). A total of 12 lines of TG mice were generated. The expression of PDX-1 was enhanced in all lines examined, assessed initially by Western blot analysis of total pancreatic protein (data not shown). These lines were examined histologically and bred to homozygosity. One highly expressing line, El-16, was used for the subsequent studies described herein.

Expression of PDX-1 in the exocrine pancreas was examined by immunostaining at e15 and e18 and postnatal days 10, 20, 30, 42, and 56. Enhanced expression of PDX-1 was observed in all epithelial cells at e15 (Fig. 1B). This contrasts with FVB control mice, which at e15 showed PDX-1 staining in a small subset of epithelial cells (Fig. 1A). By e18, all exocrine epithelial cells were strongly PDX-1 positive (Fig. 1C); by this time, PDX-1 is no longer expressed to a significant degree in control acinar cells. Staining for transgene-derived PDX-1 in the exocrine pancreas was similar in intensity to that for endogenous PDX-1 in islets. The transgene was not expressed in ducts or islets, as determined by a rat-specific PDX-1 antiserum that does not recognize mouse PDX-1 (Fig. 1D). Transgenic PDX-1 expression was maintained at all ages examined (up to age 8 months).

FIG. 2. Improved glucose tolerance and increased insulin content in El–PDX-1 TG mice. A: Intraperitoneal glucose tolerance test on EL–PDX-1 TG and FVB mice age 6 weeks. n = 8; *P < 0.05. B: Pancreas weight of TG (■) and control mice (□). C: Insulin content per pancreas in TG (■) and control mice (□) age 6 weeks. n = 6; *P < 0.05.
mice. These cells can be doublestained for insulin and NKX 6.1, a marker of mature β-cells (Fig. 1F and G), suggesting increased neogenesis of β-cells in TG mice. We also observed amylase/insulin double-positive cells at e18 in the TG mice only, suggesting that transdifferentiation could be taking place (Fig. 1H and I).

To determine whether the enhanced neogenesis of β-cells resulted in expansion of β-cell mass, we estimated the total number of β-cells and mean and total volume of β-cells. Table 1 shows the results of the stereological investigations. As a consequence of a selective decrease in the volume of the exocrine compartment (see below), the fraction of β-cell tissue per volume of pancreatic tissue was significantly increased at all ages examined; however, there was no difference in the total amount of β-cell tissue between TG and control mice at any age. In mice age 6 weeks, the total number of β-cells and the mean β-cell volume were also quantitated, and were similar between TG and control mice at any age. In mice age 6 weeks, the total number of β-cells and the mean β-cell volume were also quantitated, and were similar between TG and control mice. The frequency of large islets was much greater in TG mice than in control mice (data not shown). Large islets were also observed in the E1-1 and -3 lines (data not shown).

Increased glucose tolerance and pancreatic insulin content. The in vivo response to glucose was evaluated in TG and FVB mice age 6 weeks. No body weight differences were observed in the two groups of age-matched mice (data not shown). Fasting blood glucose was ~1 mmol/l lower in the TG mice compared with control animals (Fig. 2A). At 15 min after the injection of glucose, blood glucose values were not different between the control and TG mice. After 30, 60, and 120 min, blood glucose values declined more rapidly in the TG mice compared with the controls (P < 0.05).

Islets isolated from TG mice showed a 50% higher insulin release in response to 10 and 20 mmol/l glucose compared with FVB control mice (data not shown). To explain the enhanced in vivo and in vitro response to glucose without an increase in β-cell mass or number, we postulated that the TG mouse islets might have an increased insulin content. Indeed, despite the 60% decrease in overall pancreas weight of TG mice at this age (Fig. 2B), total insulin content per pancreas was 3.7-fold increased in TG versus control mice (6,203 ± 820.4 vs. 1,679.8 ± 748 pmol) (Fig. 2C).

Dysmorphogenesis of the exocrine pancreas. As the mice aged, the well-formed exocrine acini seen in the early pancreas became less organized and showed increasing amounts of fatty infiltration. This was observed in multiple heterozygous founders as well as in several lines that were bred to homozygosity. This dysmorphogenesis was especially noticeable at 42 and 56 days after birth (Fig. 1E). Although the amount of pancreatic tissue was already reduced at postnatal day 8, the morphologic appearance of the exocrine tissue was unaffected in day 8 mice. Beginning at 20–30 days after birth, gaps were observed within the exocrine pancreas (Fig. 1D). By age 6 weeks, and especially evident by age 8 weeks, adipose tissue appeared throughout the exocrine pancreas (Fig. 3B). By age 8
weeks, ~30–40% of the pancreatic mass had been replaced by adipose tissue. To confirm the presence of adipocytes, frozen sections were stained with Oil Red O. No Oil Red O staining was observed in the sections of the TG mouse in which the lipids had been extracted with acetone (Fig. 3B) or in the pancreas of an FVB control mouse (Fig. 3A). In contrast, there was marked staining throughout the exocrine tissue of the pancreas in TG mice age 56 days (Fig. 3C). As an independent marker of adipose tissue, pancreatic triglyceride content was measured in older control and TG mice ages 10, 20, 30, 42, and 56 days. As seen in Fig. 3D, the triglyceride content of the pancreatic extracts was greatly increased at 42 and 56 days in the TG animals, but was just above the level of detection in the control FVB animals. These observations corresponded in time to the appearance of the adipose tissue in the exocrine pancreas. Over a similar time course, multiplex RT-PCR analyses showed a dramatic increase in the expression of adipose-specific mRNAs encoding adipocyte protein-2, hormone-sensitive lipase, fatty acid synthase, and leptin in TG mice (data not shown).

We hypothesized that the observed dysmorphogenesis of acinar cells might be caused by an alteration in rates of proliferation and/or apoptosis in the TG mice. At e18, TG mice demonstrated a nearly twofold increase in BrdU incorporation in the exocrine pancreas compared with age-matched controls (Fig. 4A). The rates of replication during early postnatal days were consistently at least 20% greater in TG than in control animals. At the postweaning ages of 20, 30, and 42 days, a marked difference in replication was still apparent, as measured by MPM-2 immunoreactivity (Fig. 4B). It should be noted that BrdU labels more cells than the more specific MPM-2 antiserum. No differences in the labeling index were observed in the islets of TG versus control animals at any time point (data not shown). Many visible mitotic figures were observed throughout the exocrine tissue (data not shown). We considered the possibility that the altered architecture of the exocrine pancreas was attributable to increased apoptosis of acinar cells. By TUNEL assay, we observed a significant number of apoptotic acinar cells in the TG mice at ages 30 and 42 days (Fig. 5D), whereas few or no apoptotic cells were observed in control animals of the same age.

To further examine the ultrastructural changes in the exocrine pancreas, electron microscopy was performed on pancreases from animals ages 6 weeks and 6 months. Widespread acinar cell apoptosis was again observed in TG mice at age 6 weeks (Fig. 5C). At that age, the ultrastructure of the acinar cell in TG mice appeared, with classic exocrine granule morphology. TG mice had a larger number of degranulated cells compared with control mice (Fig. 5A and B). Colocalization of insulin and exocrinezymogen granules was not observed. At age 6 months, acinar cell size was greatly reduced. No exocrine granules were observed in the abundant adipocytes at this age.

**Changes in gene expression.** To examine changes in gene expression in the pancreases of TG mice, multiplex PCR was performed on RNA from e15 to postnatal day 10. Expression of cholesterol esterase (Fig. 6A), chymotrypsin (Fig. 6B), and amylase (Fig. 6C) was greatly decreased in the TG mice. No differences were observed in levels of the exocrine gene markers lipase, RNase, and trypsin (data not shown). Expression of duct marker genes carbonic anhydrase and cytokeratin 19 was unchanged in TG mice compared with control animals (data not shown). Insulin gene expression was already increased at e15 and e17 (Fig. 6D), as well as at postnatal days 1 and 10.

**DISCUSSION**
In these studies, we examined the effects of targeted overexpression of the transcription factor PDX-1 to the exocrine pancreas lineage using the rat elastase-1 promoter. PDX-1 is a critical regulator of pancreas development and is highly expressed in the insulin-producing β-cells of the endocrine pancreas (1–4). We postulated that PDX-1 might be sufficient to drive a program of increased β-cell development if expression was targeted to the cells destined to become the exocrine pancreas. In keeping with the initial postulate, we observed a marked
increase in the growth of the pancreas in the founder animals. In contrast, the same lines of TG mice showed decreased pancreatic size when bred to homozygosity, possibly due to a doubling of the transgene dosage. The possibility of an insertional artifact exists but is unlikely, as multiple homozygous lines analyzed showed small pancreas size and fat deposition in the pancreas.

The TG mice had an increased pancreatic insulin mRNA and peptide content and an enhanced response to glucose when compared with control mice. PDX-1 overexpression in the exocrine pancreas led to increased numbers of amylase-insulin double-positive cells (Fig. 1H), suggestive of a low level of transdifferentiation, as well as increased numbers of isolated β-cells within the acinar tissue (Fig. 1F and G), indicative of increased β-cell neogenesis. Surprisingly, islet size was increased, but overall β-cell mass was not. One explanation for the increased insulin biosynthesis and secretion in this setting is that upregulation of PDX-1 in exocrine tissue leads to expression of a signal or growth factor that stimulates insulin gene expression, biosynthesis, and secretion in the islets. Alternatively, the signal could be related to acinar cell death, as increased β-cell neogenesis has been observed in a number of pancreatic injury models (26,27).

The exocrine phenotype of these mice suggests that PDX-1 is a strong inducer of the cell cycle in the exocrine pancreas. The increase in proliferation is accompanied by a marked increase in apoptosis, leading to decreased pancreas volume and the replacement of exocrine tissue by adipose tissue. Interestingly, double TG mice that overexpress both neuroD and PDX-1 in the exocrine tissue have lower rates of proliferation, minimal apoptosis, and less adipose deposition, although glucose tolerance remains enhanced (R.S.H., unpublished observations). The expression of several exocrine-specific genes (amylase, chymotrypsin, and cholesterol esterase) was severely reduced when PDX-1 was ectopically overexpressed in the acinar cells. Because the expression of some exocrine genes was unaffected, a global loss of exocrine gene expression is not the explanation for this result. Whether this circumstance is attributable to a direct effect of PDX-1 on the promoters of these genes or a secondary effect on acinar cell differentiation is not yet clear. A recent report suggests that PDX-1 together with PBX-1b and MRG-1 (MEIS2) can act to positively regulate the exocrine elastase gene (28). Overall, the data suggest that appropriate downregulation of PDX-1 in the exocrine pancreas is required for normal acinar cell function, and that the regulation of PDX-1 in the context of the entire transcriptional milieu plays a critical role in the life cycle and differentiation state of the acinar cell.

The infiltration of the pancreas by adipose tissue has been observed in the pancreas of cystic fibrosis patients, in patients with long-standing chronic pancreatitis (G. Klöppel, personal communication), and in several other TG mouse models, including the overexpression of a dominant-negative transforming growth factor-β type II receptor in the pancreas (29). The mechanism by which
cells within the exocrine pancreas differentiate into adipose tissue is unknown. One explanation is that the PDX-1-induced proliferation of the exocrine tissue results in the expression of a factor(s) that directs fibroblasts in the pancreas to develop into adipocytes. A less likely explanation is that PDX-1-expressing exocrine cells may transdifferentiate into adipocytes.

A recent study of heterotopic expression of PDX-1 in liver in vivo demonstrated the expression of insulin in a subpopulation of hepatocytes (30). We also found a low level of acinar cells appearing to transdifferentiate to insulin-producing β-cells. The misdirected overexpression of PDX-1 in the exocrine pancreas of mice led to accelerated apoptosis of acinar cells, which were ultimately replaced by adipose tissue, suggesting that proper down-regulation of PDX-1 is critical for acinar cell survival.

ACKNOWLEDGMENTS

This work was supported in part by U.S. Public Service Grant DK-30547 (J.F.H.) and by a grant from the Danish National Research Foundation (O.D.M.). D.A.S. is a recipient of a research grant from the Juvenile Diabetes Foundation and a U.S. Public Health Service Mentored Physician Scientist Career Development Award. J.F.H. is an investigator with the Howard Hughes Medical Institute.

We thank Mette Bønding Dybaa, Holly Dressler, Lani Heller, Heather Hermann, Heidi Jensen, Ragna Jørgensen, Tove Funder-Nielsen, Tina Olsen, Jacob Steen Petersen, Erna Petersen, Susanne Sørensen, and Violeta Stanojevic for expert experimental assistance; R. J. MacDonald (University of Texas Southwestern, Dallas, TX) for the elastase promoter fragment; and H. Saski (Vanderbilt University, Nashville, TN) for the rabbit β-globin splicing and polyadenylation cassettes.

REFERENCES

10. Stoffers DS, Zinkin NT, Stanojevic V, Clarke WL, Habener JF: Pancreatic