PDX1 in Ducts Is Not Required for Postnatal Formation of β-Cells but Is Necessary for Their Subsequent Maturation

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Pancreatic duodenal homeobox-1 (Pdx1), a transcription factor required for pancreatic development and maintenance of β-cell function, was assessed for a possible role in postnatal β-cell formation from progenitors in the pancreatic ducts by selectively deleting Pdx1 from the ducts. Carbonic anhydrase II (CAII)Cre;Pdx1fl mice were euglycemic for the first 2 postnatal weeks but showed moderate hyperglycemia from 3 to 7 weeks of age. By 10 weeks, they had near-normal morning fed glucose levels but showed severely impaired glucose tolerance and insulin secretion. Yet the loss of Pdx1 did not result in decreased islet number and β-cell mass at 4 and 10 weeks of age. Within the same pancreas, there was a mixed population of islets, with PDX1 and MAFA protein expression normal in some cells and severely diminished in others. Even at 10 weeks, islets expressed immaturity markers. Thus, we conclude that Pdx1 is not necessary for the postnatal formation of β-cells but is essential for their full maturation to glucose-responsive β-cells.

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RESEARCH DESIGN AND METHODS

Animals. Transgenic mice with floxed Pdx1 (Pdx1FL/FL) (19) and constitutive CAIICre (14) were mated. In some experiments CAIICre animals carried the reporter gene from being mated with B6.129X1-ROSA26Sortm1(EYFP)Cos/J (ROSA26R.EYFP) mice from the Jackson Laboratories. DNA extracted from tails at weaning was used for genotyping with primers recognizing the floxed Pdx1 primer 5′-AGGGTTCCCGAGTGACCC-3′ and 5′-AGCAGCTG-GAGTACGGC-3′, the wild-type (WT) Pdx1 primers 5′-CTTGGACGGATCTT-3′ and 5′-GCAAACACTTGGAGATC-3′, and Cre primers 5′-GCTCGAGATTG-TTCGGATTATCT-3′ and 5′-GATCATGCATACCCAGAGA-3′. PCR was used 40 cycles for Cre, 31 cycles for floxed Pdx1, and 37 cycles for WT Pdx1 allele.

Mice were housed in the Joslin Animal Facility on a 12h light/12h dark cycle and with water and food ad libitum. CAIICre;Pdx1FL/FL mice were used for breeding to generate six genotypes: CAII Cre;Pdx1fl/fl, CAII Cre;Pdx1fl/fl, CAII Cre;Pdx1fl/fl, CAII Cre;Pdx1fl/fl, CAII Cre;Pdx1fl/fl, and CAII Cre;Pdx1fl/fl. The first two were considered higicenic experimental mice, and the others served as controls.

Body weight and morning fed glucose levels were measured weekly. Blood glucose values were measured using One-Touch glucometer (LifeScan, Milpitas, CA) on blood from tail snip. Samples for intraperitoneal glucose tolerance tests were collected from mice fasted overnight (15 h) at 0, 15, 30, 60, 90, and 120 min after an intraperitoneal injection of glucose (2 g/kg body weight). Plasma insulin was measured with a rat insulin ELISA kit (ALPCO, Salem, NH). For insulin tolerance tests, blood glucose was measured at 0, 15, 30, and 60 min after intraperitoneal insulin injection (Humulin R; Eli Lilly, Indianapolis, IN; 0.75 units/kg body weight) of fasted (9:00 a.m.–3:00 p.m.) mice.

Animals were killed under anesthesia, and the pancreas was excised for histology or islet isolation. For immunostaining, the excised pancreas was fixed for 2 h in 4% paraformaldehyde for embedding in paraffin or for frozen blocks. For secretion studies or RNA analysis, islets were isolated by the collagenase method (26), with each mouse as a separate sample for islet studies. The Joslin Institutional Animal Care and Use Committee approved all animal procedures.

Immunohistochemistry. Sections were immunostained for immunoperoxidase using the ABC kit (Vector Laboratories, Burlingame, CA) or immunofluorescence.

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Antigen retrieval was performed in 10 mmol/L citric acid buffer by microwave or PickCell 2100 antigen retriever (BD Biosciences). Sections were incubated overnight at 4°C with primary antibodies, followed by species-appropriate secondary antibodies (Supplementary Table 1). The tyramide (TSA) system (PerkinElmer, Waltham, MA) was used for amplification of PDX1, MAFA, and MAFB, following the manufacturer's instructions. Images were taken in confocal mode on a Zeiss LSM 410 microscope. For comparison of the intensity of PDX1 and MAFA staining in mice of different genotypes, images were taken at the same settings on sections from littermates stained in parallel and handled identically in Adobe Photoshop. At least three animals per genotype were examined for each antigen.

**Morphometric analysis of β- and non-β-cell mass.** Paraffin sections of 4- or 10-week-old male mouse pancreas stained by immunoperoxidase with a cocktail of non-β-cell islet hormones (glucagon, somatostatin, and pancreatic polypeptide [PP]) were analyzed by point counting morphometry for islet mass (27). β-cell mass was similarly determined on adjacent sections stained for insulin. Intersections with a 90-point grid were counted systematically in nonoverlapping fields to obtain β- and non-β-cell relative volumes (% total tissue) as well as the percentage of pancreatic parenchyma of total tissue; at least 150 fields were counted for each full footprint of pancreas section. Absolute mass was determined by multiplying the relative volume by pancreatic weight.

**Insulin secretion.** After overnight culture in RPMI 1640 medium (11 mmol/L glucose and 10% FBS), triplicate samples of 10 equilibrated islets for each mouse placed in wells of a 24-well plate were sequentially incubated with 2.6 and 16.8 mmol/L glucose in Krebs-Ringer buffer (16 mmol/L HEPES and 0.1% BSA, pH 7.4) (28,29). Supernatant fractions and cell lysates were frozen until used in the Cyquant Cell Proliferation Kit (Molecular Probes, Grand Island, NY). BSA, pH 7.4) (28,29). Supernatant fractions and cell lysates were frozen until used in the Cyquant Cell Proliferation Kit (Molecular Probes, Grand Island, NY).

**Quantitative real-time PCR.** Islets in excess of those needed for secretion were extracted for RNA using an Arcturus Picopure RNA isolation kit (Arcturus, Carlsbad, CA). After RT-PCR using a RT-PCR kit (Promega, Madison, WI), quantitative RT-PCR with SYBR green detection was performed using the ABI7300 real-time PCR system (Applied Biosystem, Foster City, CA). After RT-PCR using a RT-PCR kit (Promega, Madison, WI), quantitative RT-PCR with SYBR green detection was performed using the ABI7300 real-time PCR system (Applied Biosystem, Foster City, CA) with primers (Supplementary Table 2). Samples were normalized to ribosomal 18S, and transgenic mice followed a similar timing, 3) neither CAII nor Cre mRNA was expressed in the β-cells of the CAII<sup>F<sup>C/D<sup> mice, 4) hCAII-driven reporter at birth and Cre protein were only detected in ducts and ganglia in the pancreas, and 5) CAII<sup>F<sup>C</sup> -marked β-galactosidase background expression was about 1% of β-cells in both WT and transgenic mice (14). PDX1 protein expression that is very low to undetectable in normally quiescent adult ductal cells but has transient (3–5 days) expression after proliferation (22). Ductal cells of 4-week-old WT and CAII<sup>F<sup>C/D<sup> mice had comparable proliferation (% Ki67<sup>+</sup>) (Fig. 4F), but PDX1 protein was expressed in far fewer duct cells in CAII<sup>F<sup>C/D<sup> mice than in WT mice (Fig. 1A–D), indicating efficient excision of Pdx1 in the ducts. Because PDX1 is not expressed in pancreatic ganglia, expression of the transgene in the ganglia should have no effect on the phenotype.

**Statistical analysis.** Data are shown as mean ± SEM. For statistical analysis, an unpaired Student t test was used to compare two groups, and one-way ANOVA, followed by Bonferroni post hoc test, was used for more than two groups. A P value < 0.05 was considered statistically significant.

**RESULTS**

**Pdx1 was efficiently deleted from ducts in bigenic mice.** To test if Pdx1 expression in pancreatic ducts was necessary for islet neogenesis, we generated duct-specific Pdx1-deficient mice by mating CAII<sup>F<sup>C/D<sup> mice and Pdx1<sup>F<sup>C/D<sup> mice. Previously we showed the specificity of this promoter in that 1) CAII protein starts to be expressed in mouse pancreatic ductal cells at about embryonic day 18.5 (30), 2) lineage tracing showed the human CAII construct used in the transgenic mice followed a similar timing, 3) neither CAII nor Cre mRNA was expressed in the β-cells of the CAII<sup>F<sup>C/D<sup> mice, 4) hCAII-driven reporter at birth and Cre protein were only detected in ducts and ganglia in the pancreas, and 5) CAII<sup>F<sup>C/E<sup>D<sup> -marked β-galactosidase background expression was about 1% of β-cells in both WT and transgenic mice (14). PDX1 protein expression that is very low to undetectable in normally quiescent adult ductal cells but has transient (3–5 days) expression after proliferation (22).
CAII starts to be expressed in ductal cells only just before birth, so embryonic development was expected to be normal. The duct-specific Pdx1-deficient mice were normal in Mendelian proportion, in body weight, and morphology of the pancreas at birth (data not shown) and had nonfasting blood glucose levels within normal reference ranges over the first 2 postnatal weeks (Fig. 1E); pancreatic weight in 2-week-old littermates did not differ (control: 29.3 ± 1.0 mg; n = 4; bigenic: 31.9 ± 1.0 mg; n = 10; P < 0.16). Together these parameters indicate appropriate embryonic development.

We reasoned (Fig. 2) that if PDX1 expression in the ducts were necessary for postnatal neogenesis, neonatal formation of new β-cells from ductal precursors would be impaired in the CAII\textsuperscript{Cre}\textsuperscript{−}\textsubscript{;}Pdx1\textsuperscript{FlFl} mice, and thus, animals at 4 weeks should have an inadequate β-cell mass and be hyperglycemic (Fig. 2 option 1). By contrast, if PDX1 in the ducts were not necessary for postnatal β-cell formation, the population of β-cells at 4 weeks would include those formed before birth expressing PDX1 plus those formed from CAII promoter-driven Cre-expressing ducts after birth without PDX1 (Fig. 2 option 2).

**Impaired glucose tolerance and reduced plasma insulin in duct-specific Pdx1-deficient mice.** By weaning (Fig. 3A), the bigenic mice were moderately hyperglycemic (at 4 weeks CAII\textsuperscript{Cre}\textsuperscript{−}\textsubscript{;}Pdx1\textsuperscript{FlFl}: 254 ± 12 mg/dL, n = 23; CAII\textsuperscript{Cre}\textsuperscript{−}\textsubscript{;}Pdx1\textsuperscript{F0/F0}: 224 ± 8 mg/dL, n = 26; control: 171 ± 5 mg/dL, n = 52). Yet by 10 weeks, they had near-normal morning fed blood glucose values (CAII\textsuperscript{Cre}\textsuperscript{−}\textsubscript{;}Pdx1\textsuperscript{FlFl}: 188 ± 10 mg/dL, n = 17; CAII\textsuperscript{Cre}\textsuperscript{−}\textsubscript{;}Pdx1\textsuperscript{F0/F0}: 150 ± 5 mg/dL, n = 27; control: 153 ± 6 mg/dL, n = 33; P < 0.05 either bigenic compared with controls). Fed blood glucose values differed between CAII\textsuperscript{Cre}\textsuperscript{−}\textsubscript{;}Pdx1\textsuperscript{FlFl} and CAII\textsuperscript{Cre}\textsuperscript{−}\textsubscript{;}Pdx1\textsuperscript{F0/F0} mice only at 3 and 4 weeks of age. Unless specified, data from these genotypes are presented together as bigenic mice because we did not find differences between them. Despite near-normal blood glucose levels at age 10–11 weeks, duct-specific Pdx1-deficient mice had severely impaired glucose tolerance, as seen in intraperitoneal glucose tolerance tests (Fig. 3B), with significantly decreased plasma insulin levels (Fig. 3C) compared with the control littermates. Their ability to clear glucose in response to insulin, however, as seen in insulin tolerance tests (data not shown), did not differ. In a cohort taken to age 22 weeks, the morning fed blood glucose values of control and bigenic mice did not statistically differ from age 13 weeks onward, but there were elevated fasting glucose levels and still some impairment of glucose tolerance (Supplementary Fig. 1).

**Impaired glucose-induced insulin secretion in isolated islets of duct-specific Pdx1-deficient mice.** Islets from 11-week-old bigenic mice secreted less insulin than control islets in response to 16.8 mmol/L glucose (Fig. 3D). At high glucose, control islets secreted 0.15% of their total insulin, whereas islets from bigenic mice secreted only 0.06% of their total insulin (Fig. 3E), even though their islet insulin content was very similar (Fig. 3F). This impaired glucose responsiveness probably resulted from β-cell immaturity and a contribution from chronic mild hyperglycemia (this cohort of 11-week-old bigenic: 170 ± 6 vs. 144 ± 3 mg/dL in controls, n = 10 each group; P < 0.001), the latter known to be associated with reduced glucose-stimulated insulin secretion.

**Islet and β-cell mass of duct-specific Pdx1-deficient mice were not reduced.** These physiological data support the concept of a reduced β-cell mass at 4 weeks due to a lack of postnatal neogenesis in the absence of PDX1 in the ducts offset by some hyperglycemia-driven compensation by 10 weeks. However, we found, unexpectedly, that the islet and β-cell mass did not differ between bigenic and control male mice at age 4 or 10 weeks (Fig. 4A and B). Our technique uses a cocktail of antibodies against the non–β-cell hormones glucagon, somatostatin, and PP to allow quantification of non–β-cell and β-cell mass, so the islet peripheral mantle consisting of non–β-cells is clearly defined, and even partially degranulated β-cells are still counted. At 4 and 10 weeks, although many islets of bigenic mice had a well-defined mantle, as seen in controls, we noticed a population of islets in which core cells were immunostained with both insulin and hormone-cocktail antibodies. Immunostaining for individual non–β-cell hormones showed that the PP antibody accounted for the large number of cells coexpressing insulin and non–β-cell hormones, a notable coexpression rarely seen in postnatal control mice (Supplementary Fig. 2). We therefore quantified the β-cell mass directly on adjacent insulin-stained sections from 4-week-old male animals (Fig. 4B). Although the β-cell relative volume (% of pancreatic tissue) of bigenic mice was significantly decreased (Fig. 4C), their pancreatic weight (Fig. 4D) was increased although the animals had similar body weight (Fig. 4E). The result was that absolute β-cell mass was similar for bigenic and control animals (Fig. 4B). There was no difference in acinar or duct replication (Fig. 4F). In contrast, at age 2 weeks, although pancreatic weights did not differ among genotypes, the CAII\textsuperscript{Cre}\textsubscript{−}\textsubscript{;}Pdx1\textsuperscript{F0/F0} mice had significantly increased ductal proliferation (Supplementary Fig. 3). However, at 4 weeks (Fig. 4F-H) but not at 10 weeks (data not shown), more Ki67\textsuperscript{+} insulin\textsuperscript{+} cells were seen in islets of bigenic mice, and some of these Ki67\textsuperscript{+} cells were PDX1\textsuperscript{+} insulin\textsuperscript{+} (Fig. 4I), indicating that Pdx1-deficient β-cells can replicate.

**Mixed population of islets in duct-specific Pdx1-deficient mice, some islets having loss of key β-cell markers.** Although images for both CAII\textsuperscript{Cre}\textsubscript{−}\textsubscript{;}Pdx1\textsuperscript{FlFl} and controls were taken with the same confocal settings on parallel-processed sections, there was remarkable variation in the PDX1-immunodetection signal in insulin\textsuperscript{+} cells, even within the same section of pancreas, from 10- to 12-week-old CAII\textsuperscript{Cre}\textsubscript{−}\textsubscript{;}Pdx1\textsuperscript{FlFl} mice compared with strong homogeneous staining in control pancreas (Fig. 5A). Within
a section of CAIICre;Pdx1Fl pancreas, some islets (whether large, small or as smaller clusters) could be found containing cells with very low to undetectable PDX1 expression. Some islets had strongly homogeneous PDX1 staining, with a minority of cells displaying little or no PDX1 staining. The intensity of insulin staining also varied similarly. Thus, there was a mixed population of islets in the CAIICre;Pdx1Fl mice (Fig. 5B): about 30% had homogeneously high or normal PDX1 expression, 20% had low to undetectable expression, and 50% displayed mixed-level expression. PDX1nullinsulin+ cells accounted for $31\pm 7.7\%$ of all insulin+ cells ($n = 3$ animals with at least 18 islet/aggregates, and 625 insulin+ cells counted for each). The loss of PDX1 expression was similarly seen in the pancreas of 4-week-old
CAIICre;Pdx1Fl (Supplementary Fig. 4) and of CAIICre; Pdx1Fl/+ male mice at both ages (data not shown). When the ROSA26R-Ppys reporter gene was introduced into the CAIICre; Pdx1Fl/+ mice for lineage tracing, some lobes had YFP+ acinar and islet cells (Fig. 6A and Supplementary Fig. 5). These YFP islets have some β-cells with low to undetectable PDX1 expression, and others had strong PDX1 expression. In islets of 10- to 12-week-old mice, the β-cell transcription factor MAFA had a similarly mixed expression pattern to that of PDX1. Within the same section, some islets of the bigenic mice had little to no MAFA protein expression, in a highly heterogeneous pattern, whereas others had expression indistinguishable from controls (Fig. 6A) and islets with MAFAlow/mall were also PDX1−/− (Supplementary Fig. 6). Because MAFA has been found to be important for the functional maturation of β-cells (29), we suspected that the β-cells with low to undetectable MAFA expression were functionally immature. Increased neuropeptide Y and MAFB protein in β-cells of duct-specific Pdx1-deficient mice supports the concept of immaturity of some β-cells. Neonatal rodent β-cells lack glucose-stimulated insulin secretion (31), with a gene expression profile different from adult β-cells (32). During early development, insulin− cells express MAFA, followed by a switch to MAFA expression that can occur shortly after birth, but in adult mouse islets, the pattern resolves to MAFA expression restricted to glucagon− cells and MAFA to insulin− cells (33). Yet, in islets of 10-week-old bigenic mice, MAFA expression was detected in some insulin− cells (Fig. 7A) and in some glucagon− cells (Fig. 7B), strongly suggesting an early stage of β-cell development.

As mentioned above, the large number of cells copositive for PP and insulin were distributed throughout the pancreas. It is unlikely, however, that these cells were actually PP cells: 1) authentic PP cells are mainly localized in the head of the pancreas, 2) PP− insulin− cells are rarely seen, even in normal early stages of pancreatic organogenesis (34), and 3) importantly, most PP, peptide YY (PYY), and neuropeptide Y (NPY) antibodies cross-react (35–37). In fact, our PP antibody stained scattered cells within the colon, so it must be considered as cross-reacting with PYY (35,36). The limited selectivity of PP or NPY antibodies leads us to consider these cells as “NPY or PYY” (NPY/PYY) cells. When anti-NPY antibody was used, islets of 4- and 10-week-old bigenic mice had many insulin−NPY/PYY− and glucagon−NPY/PYY− cells in contrast to those of control mice (Fig. 7D). Bigenic mice were clearly hyperglycemic at 4 weeks, so we questioned whether the coexpression of insulin and NPY/PYY resulted from hyperglycemia. Pancreatic sections from adult rats 4 weeks after partial pancreatectomy, which showed chronic moderate hyperglycemia, had no cells with insulin−NPY/PYY− expression (Fig. 7E), indicating that induction of NPY/PYY expression in β-cells was not caused by hyperglycemia. Recently, NPY expression was reported in adult insulin− cells after embryonic-stage β-cell–specific deletion of NeuroD1, and these cells were characterized as immature β-cells based on expression of NPY and lactate dehydrogenase A.
In our study, insulin+ cells with low levels of PDX1 and MAFA expression, coexpressing MAFB and NPY/PYY seen in duct-specific Pdx1-deficient pancreas, strongly suggest that the β-cells formed postnatally remained immature, even at 10 weeks of age. Decreased expression of β-cell functional genes and increased expression of immature β-cell markers in islets of duct-specific Pdx1-deficient mice. Consistent with our immunostaining findings, insulin, Pdx1, and mafa mRNA levels were significantly lower in islets of 11-week-old duct-specific Pdx1-deficient mice than in controls (Fig. 7E). Increased gene expression of both mafb and LDHA, the latter not expressed in adult β-cells but expressed (in rat islets) up to about 1 week postnatally (39), is consistent with our conclusion of the functional immaturity of these islets. Importantly, PYY mRNA was elevated in islets of duct-specific Pdx1-deficient mice compared with controls, in contrast to PP and NPY mRNA.

**DISCUSSION**

By specifically deleting Pdx1 from pancreatic ducts using duct-specific Cre-lox methods, we showed that β-cell development occurs even in the postnatal absence of PDX1 in ducts but that the resultant neogenetic insulin-PDX1-null cells have characteristics of immature β-cells. Thus, we are able to arrive at the significant conclusion that Pdx1 is not necessary postnatally for formation of β-cells but is necessary for their full maturation to glucose-responsive β-cells. It is especially interesting that some islets, even within the same section, showed strong heterogeneity, with most β-cells PDX1-deficient, yet other islets showed uniformly strong PDX1 staining. These extremes probably represent, respectively, populations of newer postnatal islets and older prenatally formed islets. Importantly, we speculate that the presence of some islets with mostly strong uniform PDX1 staining, with small numbers of cells showing little or no PDX1 signal, could represent newly formed β-cells migrating to and coalescing with older islets.
Contrary to our initial hypothesis that duct-specific deletion of Pdx1 would limit postnatal islet neogenesis and result in lower islet mass at 4 weeks, with a possible "compensatory rebound" resulting from increased replication by 10 weeks, our data show that islet and β-cell mass were normal in the duct-specific Pdx1-deficient mice, with at least 30% of the β-cells lacking PDX1 protein. The lineage of such cells was verified by eYFP expression of the lineage marker. Thus, we conclude that new β-cells are able to form, in true neogenetic fashion, from postnatal ducts in which Pdx1 function is prevented. The finding that pancreatic weights were increased in bigenic mice at age 4 weeks but not at age 2 weeks was puzzling. In control mice, this 2-week period is one of an extensive expansion of the pancreas (three- to fourfold increase, from 29.3 to 110.2 mg). In bigenic mice at 2 weeks, ductal proliferation was increased above the already high level of controls, whereas at 4 weeks, the proliferation of the exocrine pancreas (acinar and duct) was similar to the controls. Analyses of Pdx1 tet-off inducible mouse model (40,41)

![Image of islets with lineage tracing marker and low to undetectable MAFA expression.](image-url)

**FIG. 6.** Islets with PDX1null β-cells show lineage tracing marker and low to undetectable MAFA expression. A: The variation of PDX1 immunostaining corresponded with the expression of lineage marker YFP in islets from a 4-week-old CAIICre;Pdx1Fl/fl (blood glucose: 278 mg/dL) mouse. The middle panel shows YFP expression as split green channel of images shown in the top panel (insulin, red; YFP, green). The bottom panel shows same islets on adjacent section (due to antibody compatibility issues) with PDX1 (green) and insulin (red). a, lineage-marked acinar cell. *Identifies the same cell in different images. B: MAFA expression (green) showed similar variation from high intensity to low/undetectable in insulin (red) islets from same section of a 10-week-old CAIICre;Pdx1Fl/fl mouse (blood glucose at 4 weeks: 272 mg/dL, 10 weeks: 189 mg/dL) compared with homogeneous high intensity of control littermate (blood glucose at 4 weeks: 172 mg/dL, 10 weeks: 178 mg/dL).
FIG. 7. Islets of 10- to 11-week-old bigenic mice expressed markers of immature β-cells. A and B: MAFB protein (green) was restricted to glucagon+ cells (red) in adult control (c) islets, but in bigenic (Pdx1<sup>Cre<sup>) there were both glucagon+ cells (red) and insulin+ cells (red) that were MAFB+. The insets in the bigenic images show higher magnification of positive cells with DAPI-stained nuclei. In bigenic mice (c) (here blood glucose at 4 weeks: 254 mg/dL, 10 weeks: 145 mg/dL), many insulin+ cells (green) and some glucagon+ cells (green) coexpressed NPY/PYY (red), whereas in controls (D) (here blood glucose at 4 weeks: 162 mg/dL, 10 weeks: 156 mg/dL), only some glucagon+ cells coexpressed NPY/PYY (red). The same islets from adjacent sections are shown for insulin/NPY and glucagon/NPY immunostaining for bigenic and controls. E: Quantitative PCR for selected genes on RNA from islets of the same 11-week-old animals as used for insulin secretion (Fig. 3D–F) showed significant decreased expression of insulin, pdx1, and mafa mRNA and significant increased expression of PYY, mafb, and LDHA mRNA in bigenic mice (■), shown normalized to controls (□, n = 7–9). Data are mean ± SEM. *P < 0.05.

showed that repression of Pdx1 had very different results dependent on its timing. If Pdx1 repression were initiated in mid-embryonic stage, acinar differentiation was impeded, but if initiated in the adult, exocrine (acinar and duct) proliferation was stimulated. Our data indicate that during the neonatal period of rapid pancreatic expansion, the lack of Pdx1 in the ducts resulted in a greater proliferation of duct cells that gave rise to more acinar cells and greater pancreatic weights.

With the current strong controversy over whether pancreatic ducts can give rise to new islet cells or even acinar cells postnatally (1), it is relevant to consider alternative explanations to our current findings. Could there be misexpression of carbonic anhydrase II, and thus Cre recombinase expression, in β-cells? CAII is normally expressed in rodent glucagon-expressing α-cells but not β-cells (30). In the experiments reported here, we used the human CAII promoter because CAII is limited to ducal expression in humans, and Cre immunostaining in the CAII<sup>Cre<sup> pancreas was only seen in ducts and ganglia (14). With no injury involved in the current study, any misexpression would have to be significant to result in 30% labeled β-cells. Previously, however, we reported that even 48 cycles of RT-PCR failed to detect Cre or CAII mRNA in fluorescence-activated cell sorted β-cells from day 1, 2, 4, or 8-week-old CAII<sup>Cre<sup>;MIP<sup>Cre<sup> mice but was easily detected in the kidneys from the same animals (14). The isolated islets used in the current study had no detectable Cre mRNA expression by quantitative PCR.

The glucose intolerance of the bigenic mice showing 70% of the β-cells as “immunofluorescently normal” was unexpected because rodents with 60% partial pancreatectomy maintain normal glucose homeostasis. Regeneration and adaptation have been found in mice and rats after 60% partial pancreatectomy, seen as the 40% β-cell mass of the remnant increasing to about 55% of sham controls (42,43) with an accompanying increase in function of individual β-cells (44,45). One must consider that the reduced glucose responsiveness partly results from glucotoxicity because chronic mild hyperglycemia was present from at least 3 weeks of age in these mice. Even slightly increased (15–20 mg/dL) blood glucose levels for at least 6 weeks can result in impaired glucose-responsive insulin secretion (42) and large alterations in gene expression (46). In our case, it is still unclear why hyperglycemia began at between 2 and 3 weeks of age. Lineage tracing experiments have suggested substantial de novo β-cell formation during this period (47). Moreover, studies of β-cell maturation in neonatal rats (13,31,32,48) show that 3-week-old pups are transiently insulin-resistant and that their β-cells are not functionally mature. In this context, a large functional impairment in 30% of the β-cells may result in modest hyperglycemia.

The presence of several markers of immature β-cells suggests that functional immaturity is partly responsible for the lack of glucose responsiveness of the isolated bigenic islets. In islets from duct-specific Pdx1-deficient mice, mafa mRNA and protein had lower than normal
expression for adult β-cells, being similar to those in neonatal β-cells (29). We previously showed that although mafa overexpression could induce the maturation of glucose-responsiveness in neonatal islets, Pdx1 overexpression could not within the experiment's timeframe (29). However, PDX1high is expressed before MAFA in insulin+ cells during development (33), suggesting that Pdx1 is an upstream regulator of mafa; thus, we expect that with longer incubation, Pdx1-infected P2 islets would have induced mafa expression and subsequently acquire glucose responsiveness. Furthermore, mafb, LDHA, and PYY mRNA were more highly expressed in bigenic islets compared with control. We conclude that the increased mafb mRNA did not reflect an increased proportion of glucagon-expressing cells, because the islet and β-cell mass were unaltered. The continued coexpression of MAFB (which is normally extinguished in mouse β-cells) and insulin in adult bigenic mice suggests that those cells remained in an early stage of β-cell development (33). Isolated islets of adult Pdx1-deficient mice also had elevated LDHA mRNA, another gene highly expressed in immature islets (39) but hardly expressed in normal adult β-cells (39,49) and induced by chronic hyperglycemia (50). Taken together, the increased expression of NPY/PYY, mafb, and LDHA and low mafa in β-cells suggest that PDX1 is necessary for the full maturation of β-cells.

We conclude that PYY is likely the specific member of the NPY/PYY/PP family that is aberrantly expressed in the duct-specific Pdx1-deficient β-cells. The cross-reactivity of most PP, PYY, and NPY antibodies has probably contributed to several previously apparently discordant conclusions. PYY and NPY were reported as markers of immature β-cells when coexpressed with insulin (34,36,38,51) and PYY as a marker of early islet precursors (35,36). After birth, NPY expression in pancreatic islets was reported as restricted to neonatal β-cells and absent from adult β-cells (52). Recently, however, NPY was reported in adult-stage insulin+ cells after embryonic β-cell-specific deletion of NeuroD1, and these cells were classified as immature based on expression of NPY protein/mRNA, LDHA, and lack of glucose-responsiveness (38). In our bigenic genetic manipulation, a large number of insulin+NPy+PYY+ cells were detected in islets, but mRNA for only PYY, not NPY or PP, was increased in islets from 11-week-old bigenic mice compared with controls. The discrepancy of NPY mRNA islets, but mRNA for only PYY, not NPY or PP, was increased in islets from 11-week-old bigenic mice compared with controls. The discrepancy of NPY mRNA with the cross-reporting of models of embryonic development. Diabetes 1993;42:1715–1720

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