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Context-specific α-to-β-cell reprogramming by forced Pdx1 expression

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Using single transcription factors to reprogram cells could produce important insights into the epigenetic mechanisms that direct normal differentiation, or counter inappropriate plasticity, or even provide new ways of manipulating normal ontogeny in vitro to control lineage diversification and differentiation. We enforced *Pdx1* expression from the Neurogenin-3-expressing endocrine commitment point onward and found during the embryonic period a minor increased β-cell allocation with accompanying reduced α-cell numbers. More surprisingly, almost all remaining Pdx1-containing glucagon/Arx-producing cells underwent a fairly rapid conversion at postnatal stages, through glucagon–insulin double positivity, to a state indistinguishable from normal β cells, resulting in complete α-cell absence. This α-to-β conversion was not caused by activating Pdx1 in the later glucagon-expressing state. Our findings reveal that Pdx1 can work single-handedly as a potent context-dependent autonomous reprogramming agent, and suggest a postnatal differentiation evaluation stage involved in normal endocrine maturation.

Supplemental material is available for this article.

Received April 27, 2011; revised version accepted July 5, 2011.

A major hurdle for cell replacement-based diabetes therapy is the difficulty of supplying vast numbers of functioning insulin-producing β cells. One method could be through the reprogramming of alternative cell types. While this process might be easier with closely lineage-related cells, even substantially different cells may be susceptible (e.g., Zhou et al. 2008).

Recent studies reveal significant plasticity between pancreatic α and β cells under certain induced conditions, implying a potential route to β cells through α cells. In a near-total β-cell destruction and regeneration model in adult mice, a proportion of new β cells were produced from α cells via a bihormonal glucagon$^+$insulin$^+$ (Gcg$^+$Ins$^+$) transitional state (Thorel et al. 2010). The interconversion presumably occurs in response to a combination of the physiological need to replenish β cells and regeneration-induced stress, raising questions as to the local or systemic signals triggered by such lesions. Direct superimposition of a pro-β-lineage condition was reported when *Pax4* expression was forced in pancreatic or endocrine progenitors or in embryonic α cells to redirect endocrine differentiation or coax pre-existing α cells into β cells. The converted cells seemed similar to normal β cells and temporarily improved glycemia under induced diabetes, although the effect was superseded by uncontrolled α-cell neogenesis and fatality caused by extreme hyperglycemia (Collombat et al. 2009). These studies on the ability of a single lineage-allocating transcription factor to sustain complete cell fate conversion suggest that similar analyses for other transcription factors could be insightful. Determining which factors induce specific types of lineage reprogramming, as well as the repertoire of cellular competence states amenable to fate switching, could lead to pharmacological intervention to activate such factors in vivo, or to improved differentiation of embryonic stem cells to β cells.

Clues to the fate-instructing capacity of *Pdx1* as a β-cell selector are inferred from its enriched expression in embryonic and mature β cells. Ectopic *Pdx1* alone can induce incomplete reprogramming of liver or pancreatic acinar cells (e.g., Ferber et al. 2000; Heller et al. 2001). A synergistic effect between Pdx1, Neurog3, and MafA was observed when acinar cells were converted into β-like cells (Zhou et al. 2008), which inefficiently ameliorated hyperglycemia caused by loss of endogenous β cells, perhaps because the reprogrammed cells did not assemble into islet-like clusters. Rather than triggering a redirection into endocrine cells, forced *Pdx1* expression in *Ptf1a*-expressing cells caused late stage acinar-to-ductal hyperplasia (Miyatsuka et al. 2006). While these studies suggest that *Pdx1* alone is contextually sufficient to induce partial *trans*-differentiation or *trans*-determination, little is known about how different competence states affect the response to this single factor.

Here, we report on the previously unknown sufficiency for *Pdx1* as a potent regulator of endocrine lineage allocation and maintenance of the mature state. With Pdx1 expression enforced from the Neurog3$^+$ endocrine progenitor state onward, two periods of dominant lineage redirection occurred: (1) during early organogenesis, a minor reproducible reduction in cells directed to the α fate, and (2) a surprising peri/postnatal redirection of Pdx1-expressing α cells, with rapid reprogramming into Ins$^+$ cells that are indistinguishable from normal β cells. The delayed conversion occurred despite α cells having expressed exogenous Pdx1 from their endocrine commitment point onward, suggesting the possibility of a cryptic chromatin-priming effect. In contrast, exogenous Pdx1 in Gcg$^+$ embryonic or adult α cells suppressed Gcg expression but did not induce α/β fate switching. Our findings reveal differential α-to-β plasticity between endocrine progenitors and hormone-secreting cells in response to *Pdx1*. We speculate on the epigenetic ramifications of these differential lineage-switching findings.

[*Keywords*: pancreas; endocrine progenitors; α and β cells; reprogramming; Pdx1]
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Article is online at http://www.genesdev.org/cgi/doi/10.1101/gad.16875711.

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Results and Discussion

Exogenous Pdx1 *expression in endocrine progenitors*

Forced "constitutive" *Pdx1* expression was derived from a *CAG-CAT-Pdx1* allele (Miyatsuka et al. 2006) via a BAC transgene driving Cre from *Neurog3* regulatory elements ($Neurog3^{TgBAC\text{-}Cre}$) (Schonhoff et al. 2004). $Neurog3^{TgBAC\text{-}Cre}$-mediated *CAT* excision led to Flag-tagged Pdx1 (^{Flag}Pdx1) production in Neurog3$^+$ descendants from the ubiquitously active *CAG* promoter (Fig. 1A). We compared tissues from $Neurog3^{TgBAC\text{-}Cre}$;*CAG-CAT-Pdx1*; $R26R^{EYFP}$ mice (referred to as $Neurog3^{Cre}$-$Pdx1^{OE}$ hereafter) with those from $Neurog3^{TgBAC\text{-}Cre}$;$R26R^{EYFP}$ littermate controls.

Two key criteria were evaluated for *Neurog3*-dependent activation of *CAG-CAT-Pdx1*. First, selective ^{Flag}Pdx1 production within endocrine cells was confirmed by Flag immunostaining, with signal only in $Neurog3^{Cre}$-$Pdx1^{OE}$ pancreas, from embryonic day 16.5 (E16.5) to postnatal stages (Fig. 1B–E). Second, ^{Flag}Pdx1 immunodetection with Pdx1 antibodies labeled cell types that normally do not express Pdx1 at high levels (Pdx1^{HI}). A large increase occurred in the number of Pdx1^{HI} cells in E14.5 $Neurog3^{Cre}$-$Pdx1^{OE}$ pancreatic epithelium compared with equivalent control tissue (Fig. 1F,G). Ectopic Pdx1 was detected in non-β/non-δ endocrine cells (i.e., in α, PP, and ε cells). We found Pdx1^{HI} Gcg$^+$ α cells in postnatal day 1 (P1) $Neurog3^{Cre}$-$Pdx1^{OE}$ pancreas, while control α cells were Gcg$^+$Pdx1$^-$ (Fig. 1H,I). This evidence demonstrates a spatiotemporally defined system for endocrine–progenitor-selective exogenous *Pdx1* expression in the $Neurog3^{Cre}$-$Pdx1^{OE}$ pancreas.

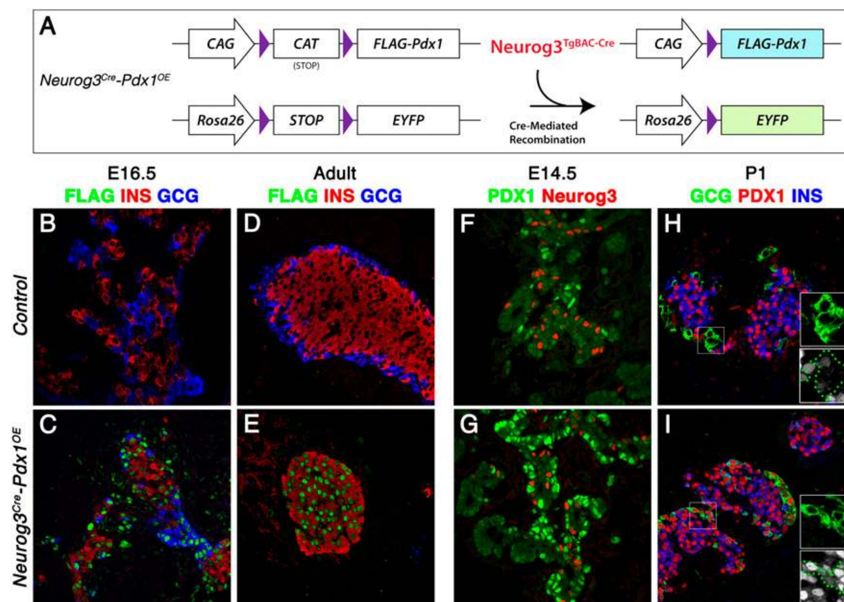

Figure 1. Neurog3^{Cre}-mediated exogenous Pdx1 expression. (*A*) Schematic presentation of *CAG-CAT-Pdx1* and $R26R^{EYFP}$ and Cre recombination. Exogenous Flag-tagged Pdx1 (Flag-Pdx1) and EYFP expression is activated after CAT or STOP cassette excision. (*B–E*) ^{Flag}Pdx1 was only detected in the $Neurog3^{Cre}$-$Pdx1^{OE}$ pancreas. No Gcg was detected in adult $Neurog3^{Cre}$-$Pdx1^{OE}$. (*F*,*G*) Increased Pdx1$^+$ cells were detected in E14.5 $Neurog3^{Cre}$-$Pdx1^{OE}$ compared with control. (*H*,*I*) Ectopic Pdx1 in Gcg$^+$ cells in $Neurog3^{Cre}$-$Pdx1^{OE}$ but not control (dashed lines in *insets*; white, Pdx1).

Progressive reduction of α cells in the Neurog3^{Cre}-Pdx1^{OE} *pancreas*

Global glucose homeostasis and the overall size and outward appearance of the pancreas in $Neurog3^{Cre}$-$Pdx1^{OE}$ mice were similar to controls (Supplemental Fig. 2). However, a drastic Gcg$^+$ cell loss (>99%) was found in the adult pancreas, while other hormone-producing cell types were similar in number and location (Fig. 1E; Supplemental Fig. 1). The numbers of α (Gcg$^+$) or β (Ins$^+$) cells were quantified at key stages. At each stage, the combined number of α and β cells ("α + β") was similar between $Neurog3^{Cre}$-$Pdx1^{OE}$ and control, despite a significant difference in the α versus β representation (Fig. 2I; Supplemental Fig. 3). This observation strongly suggests a scenario of lineage diversion, wherein one cell population expands at the expense of the other under a constant total number. We identified two phases of lineage conversion that ultimately contributed to a complete α-cell loss by the early adult stage. First, a significant decrease in Gcg$^+$ cell number (control 47% representation reduced to 35%) and accompanying increase in Ins$^+$ cells was detected in the E16.5 $Neurog3^{Cre}$-$Pdx1^{OE}$ pancreas, shortly after the peak of *Neurog3* expression at approximately E15 (Gu et al. 2002). Coexpression of Ins and α-cell-specific factors, such as Arx, suggesting an early movement toward β-cell-directed *trans*-differentiation, was not detected at this stage (data not shown). The early phase shift in α/β representation suggests that exogenous *Pdx1* biases the behavior of a fraction of early endocrine progenitors, increasing flux toward the β lineage, disfavoring the α lineage. The reason for only 25% of Gcg$^+$ cells being affected could be related to nonuniformity in Pdx1 accumulation within Neurog3$^+$ progenitor cells and/or their immediate progeny.

Second, a major progressive loss of Gcg$^+$ cells concurrent with increased Ins$^+$ numbers was detected at P1–P12 (Fig. 2I). The number of Gcg$^+$ cells in the P1 $Neurog3^{Cre}$-$Pdx1^{OE}$ pancreas was 32% of controls, suggesting that loss of *Gcg* expression in some cells had been initiated around birth. By P12, few Gcg$^+$ cells were present, which were usually Gcg^{LO} by immunodetection (Fig. 2H). Importantly, numerous mantle-located Gcg$^+$Ins$^+$ cells were detected (Fig. 2D,F), representing intermediate state α cells undergoing conversion. The presence of Gcg$^+$Ins$^+$ cells in $Neurog3^{Cre}$-$Pdx1^{OE}$ suggests that suppression of *Gcg* and induction of *Ins* occurred concurrently. Consistently, we did not detect Synaptophysin$^+$ endocrine cells that were not producing islet hormones (Pdx1^{HI}Gcg$^-$Ins$^-$ cells) (data not shown). We therefore propose that the large numbers of Pdx1^{HI} α cells produced after endocrine specification (Pdx1^{OE} via *Neurog3* promoter activity), but only after a considerable delay until the peri/postnatal period, undergo a remarkable transformation toward Ins-expressing cell types.

Further validation by quantitative RT–PCR (qRT–PCR) of pancreatic tissue ($Neurog3$-$Pdx1^{OE}$ islets could not be isolated) (see Supplemental Fig.

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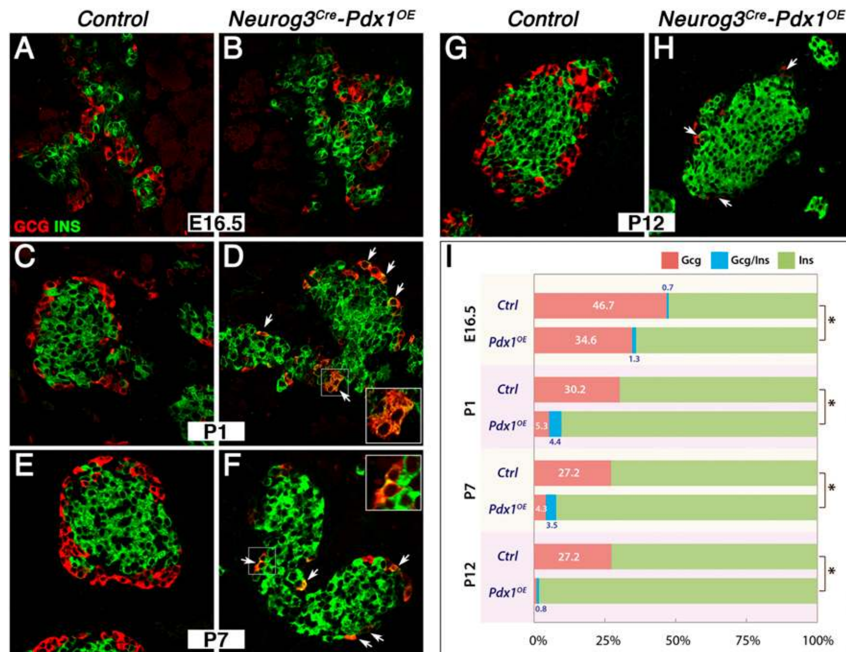

Figure 2. Progressive decrease of Gcg$^+$ cells concomitant with increase of Ins$^+$ cells. (*A–H*) Ins and Gcg expression at E16.5, P1, P7, and P12. (Arrows) Gcg$^+$Ins$^+$-coexpressing cells. (*I*) Quantitative analysis of cells with Gcg, Ins, or Gcg-Ins expression in control and *Neurog3^{Cre}-Pdx1^{OE}*; (*) $P < 0.05$.

4),examining a panel of differentiation pathway transcriptional effectors and hormones, confirmed the postnatal alteration in α/β-cell proportions. Expression of *Gcg* and *Pou3f4*, encoding an α-cell factor involved in *Gcg* transactivation (Hussain et al. 1997), was significantly reduced and increased *Ins* and *Nkx6.1* expression was detected with increased *Pdx1* expression (Supplemental Fig. 4). The considerable increase in *Sst* and *Ppy* RNA in *Neurog3^{Cre}-Pdx1^{OE}* tissue was not associated with an overt difference in δ- or PP-cell numbers, or coexpression of Ins with Sst or Ppy, but could represent increased expression per cell (Supplemental Fig. 1).

It was important to address whether the loss of Gcg$^+$ α cells in *Neurog3^{Cre}-Pdx1^{OE}* might be caused by the death of Pdx1^{HI} α cells or overproliferation of other endocrine cells outcompeting or stifling Pdx1^{HI} α cells. Any dying cells should have been relatively easily detected in the mantle location over the P1–P14 postnatal reprogramming period. No difference was observed in general apoptosis or cell proliferation (TUNEL- or BrdU/Ki67-labeling assay) between *Neurog3^{Cre}-Pdx1^{OE}* and control (Supplemental Fig. 5), supporting the hypothesis that Pdx1^{HI} α cells become actively reprogrammed via a Gcg$^+$Ins$^+$ transitional state into β cells. The comparable α + β population size between genotypes at all stages also supports our interpretation of a Pdx1-induced delayed reprogramming at the perinatal hormone-expressing stage, with no net loss of total endocrine cells.

Postnatal completion of α-to-β reprogramming induced by Pdx1

Ideally, pulse-chase lineage tracing of α-cell progenitors, with specific labeling concurrent with or just following the *Neurog3*$^+$ state, should unequivocally demonstrate the proposed α-to-β-cell conversion. Unfortunately, we could not perform Cre-based lineage tracing on top of our Cre-based Pdx1 activation method, and a suitable genetic tool for this test (i.e., an *Arx^{CreER}* strain) is not available. We therefore directly examined the production of Arx, a crucial α-cell transcription factor (Collombat et al. 2003), as an alternative approach to evaluate the α-cell reprogramming. In P1 *Neurog3^{Cre}-Pdx1^{OE}*, all Gcg$^+$ cells, which expressed Pdx1 ectopically (Fig. 1I), were Arx$^+$, as in controls (Fig. 3B). The total number of Arx$^+$ cells and the *Arx* mRNA level were comparable with controls at P1 (Fig. 3B; Supplemental Fig. 4). The presence of both Arx$^+$Gcg$^+$ and Arx$^+$Ins$^+$ cells is most consistent with the idea that normal numbers of α cells were initially produced and that the reprogramming was far from complete at birth. Arx was also present in the Gcg$^+$Ins$^+$ cells over the postnatal period. In adult *Neurog3^{Cre}-Pdx1^{OE}* tissue, we reproducibly detected Arx in scarce, mantle-located Ins$^+$ monohormonal cells. These Arx$^+$Ins$^+$ cells were detected in few islets, although sometimes several per islet, and were absent in controls (Fig. 3D). The Ins signal in these cells was similar to the other Ins$^+$ cells. Intriguingly, the decrease in total Arx$^+$ cell number and mRNA level became apparent only at P7–P12, while the Gcg$^+$ cell number and the mRNA level were already reduced by P1 (Fig. 2I; Supplemental Figs. 4, 6). The conversion of hormone expression (Gcg to Ins) seems to precede down-regulation of the α-cell progenitor factor Arx,

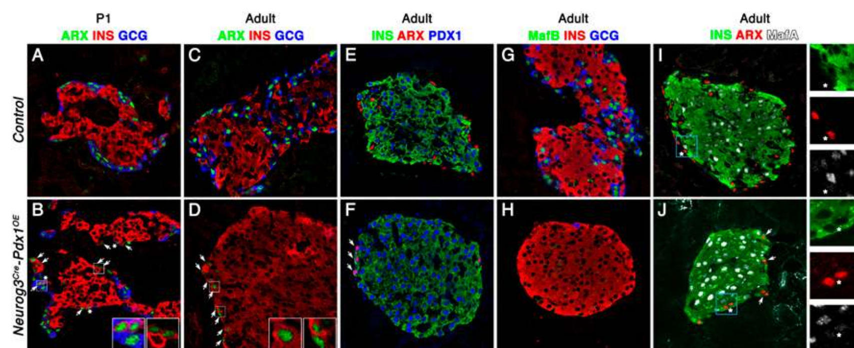

Figure 3. Loss of α-cell character in the *Neurog3^{Cre}-Pdx1^{OE}* pancreas. (*A–F*) Arx was expressed in Gcg$^+$ cells in control in P1 (*A*) and adult pancreas (*C*). In *B*, Arx was found in Gcg$^+$, Gcg$^+$Ins$^+$ (asterisks), and some Ins$^+$ cells in P1 *Neurog3^{Cre}-Pdx1^{OE}* (arrows, respectively). Only a few islet mantle Ins$^+$ cells expressed Arx in adult *Neurog3^{Cre}-Pdx1^{OE}* (arrows in *D* and *inset*). They also expressed Pdx1^{HI} (arrows in *F*). (*G,H*) MafB was detected in Gcg$^+$ cells in the control adult pancreas but not *Neurog3^{Cre}-Pdx1^{OE}*. (*I,J*) Most Ins$^+$ cells, except for a few Arx$^+$Ins$^+$ cells (arrows; also asterisks and separated channels), expressed MafA in *Neurog3^{Cre}-Pdx1^{OE}* adult at levels comparable with control.

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with most $Pdx1^{HI}$ α cells acquiring a more completely reprogrammed state by P7–P12. Despite their small number, the presence of adult $Arx^{+}Ins^{+}$ cells with apparently normal Ins immunodetectability per cell agrees with the idea that Pdx1 is dominant over Arx in determining the overt hormone-expressing state, even when in direct competition with the α-cell determinant.

Consistent with the massive postnatal reprogramming of *Neurog3*^{Cre}-*Pdx1*^{OE} α cells, a decrease in $MafB^{+}$ cells in P12 and adult pancreas (Fig. 3H; Supplemental Fig. 6) confirmed the lack of normally differentiated mature α cells and the absence of MafB in Ins^{+} cells (whether reprogrammed or not). Accordingly, a large decrease in *MafB* mRNA was found in P12 *Neurog3*^{Cre}-*Pdx1*^{OE} (Supplemental Fig. 4). Besides its endocrine progenitor function, *Pax6* was implicated in the control of the transcription of several α-cell-associated genes, such as *Gcg* and *MafB* (Gosmain et al. 2010). Interestingly, *Pax4* could repress *Pax6*-mediated transactivation in vitro (Ritz-Laser et al. 2002). We speculate that the *Pax6* reduction at P12 (Supplemental Fig. 4) results indirectly from the ectopic Pax4 in Gcg^{+} cells, described below.

Next, we assessed whether a complete β-cell differentiation program was deployed in the reprogrammed α cells. MafA, a marker associated with "final maturation" of β cells (Artner et al. 2010), at a level comparable with that in control Ins^{+} cells, was found in all Ins^{+} cells in *Neurog3*^{Cre}-*Pdx1*^{OE} tissue, irrespective of core or peripheral location. The rare adult $Arx^{+}Ins^{+}$ cells were always $MafA^{-}$ (Fig. 3J), suggesting that these former α cells resist complete reprogramming. Equivalent results were found for two more β-cell maturity markers: Nkx6.1 and Glut2 (Supplemental Fig. 7; Guillam et al. 1997; Sander et al. 2000). All of our evidence is therefore consistent with the idea that the vast majority of $Pdx1^{HI}$ α cells present at the perinatal stage undergo reprogramming toward authentic β cells, exhibiting both a normal mature state and complete loss of preceding α-cell identity. *Pdx1* is dominant over *Arx* with respect to driving cells toward an $Ins^{+}Gcg^{-}$ state, and Arx repression is prerequisite for thorough reprogramming.

The qRT–PCR analysis on postnatal *Neurog3*^{Cre}-*Pdx1*^{OE} pancreas showed significant up-regulation of genes encoding β-cell factors (*MafA*, *Nkx6.1*, and *Pax4*) (Supplemental Fig. 4), likely caused by the increased number of converted β cells (Fig. 2I) as well as the additional exogenous *Pdx1* expression within all β cells. We note a likely normal function of these β cells, because the mice had normal to slightly decreased glycemia (the latter reflecting the lack of counterregulatory Gcg), and glucose tolerance analogous to controls (Supplemental Fig. 2). Pax4, previously reported to function as an α-cell reprogrammer (Collombat et al. 2009), was ectopically expressed in a proportion of $Pdx1^{HI}$ α cells (Supplemental Fig. 6). While *Nkx6.1* expression was directly correlated with increased Pdx1 (e.g., at P1), *Pax4* showed a late response (increased by P12 but not P1). This *Pax4* pattern complements the graded decrease of *Arx* (Supplemental Fig. 4), agreeing with their proposed antagonistic relationship (Collombat et al. 2003). We propose that the initial hormone switching, which begins at P1 and precedes *Pax4* elevation, could be Pax4-independent. It will be important to determine whether Pdx1 initiates a postnatal induction of Pax4, and whether their mutual reinforcement of expression works in a synergistic feed-forward loop to engage full reprogramming. Notably, *Pdx1* overexpression triggered postnatal α-to-β reprogramming via an intermediate cell type ($Ins^{+}Gcg^{+}$) and did not lead to oversized islets, profound α-cell neogenesis, and failure to maintain euglycemia (Supplemental Figs. 2, 3), phenotypes therefore distinct from those caused by *Pax4* manipulation (Collombat et al. 2009). On the other hand, exogenous *Pax4* was sufficient to coax Gcg^{+} embryonic α cells into β cells while *Pdx1* was not. Further study could address the detailed gene regulatory network responses to Pax4 and Pdx1.

Collectively, our data show that the majority of $Pdx1^{HI}$ α cells were successfully reprogrammed toward a mature β-cell profile. While *Gcg* and *Pou3f4* expression was rapidly reduced, we believe that it is the repression of *Arx* in α cells that allows full-scale transformation toward an authentic β-cell program. Although further analyses will be required to assess the early-acting effects of $Pdx1^{HI}$ that are imposed at the early $Neurog3^{+}$ state and/or continued during islet α-cell differentiation, we hypothesize that an early progenitor period priming is crucial for the later (peri/postnatal) override of the *Arx*-induced α-cell program. The peri/postnatal fate conversion could reflect a final fate assessment and switching of cellular maturation (with dominant establishment of *Pdx1*-centered regulatory networks) in response to the early, but initially cryptic, establishment of a $Pdx1^{HI}$ condition in developing α cells. With this reasoning, we asked whether the effect noted when expressing $Pdx1^{HI}$ from the endocrine progenitor state onward was different if $Pdx1^{HI}$ expression was initiated within cells slightly further along the differentiation pathway, having already started *Gcg* expression.

Glucagon suppression but incomplete reprogramming of embryonic and mature α cells

Activating the $Pdx1^{HI}$ condition in embryonic or adult α cells produced evidence that embryonic α cells have already acquired resistance to reprogramming. Because exogenous *Pdx1* selectively affected the α lineage, we first tested the effect of *Pdx1* overexpression in embryonic Gcg^{+} cells. *Gcg*^{TgCre} mice (Herrera 2000) were used to generate *Gcg*^{TgCre};*CAG-CAT-Pdx1;R26R*^{EYFP} mice (*Gcg*^{Cre}-*Pdx1*^{OE} hereafter), driving exogenous *Pdx1* expression in Gcg^{+} cells, with *Gcg*^{TgCre};*R26R*^{EYFP} littermate controls. Similar lineage-labeled YFP^{+} cell numbers were detected within the islet mantle of *Gcg*^{Cre}-*Pdx1*^{OE} adult pancreas and controls (aged 3–3.5 mo). In *Gcg*^{Cre}-*Pdx1*^{OE}, but not control, exogenous $Pdx1^{HI}$ was detected in a majority of lineage-labeled α cells (~56% $YFP^{+}Pdx1^{HI}$ cells among all YFP^{+} cells, compared with 0% in control) (Fig. 4I), possibly reflecting nonparallel recombination of *R26R*^{EYFP} and *CAG-CAT-Pdx1*. While Gcg was not detected in most (>94%) $YFP^{+}Pdx1^{HI}$ cells, only a low number (<14%) showed any Ins signal, thereby leaving ~90% of $Pdx1^{HI}$ α cells expressing neither hormone ($YFP^{+}Pdx1^{HI}Gcg^{-}Ins^{-}$ cells) (Fig. 4K). Therefore, despite these Gcg^{+} cells having expressed $Pdx1^{HI}$ for roughly the same period as those in *Neurog3*^{Cre}-*Pdx1*^{OE} (late embryogenesis through adult), the α-to-β conversion in *Gcg*^{Cre}-*Pdx1*^{OE} was dramatically less efficient than that in *Neurog3*^{Cre}-*Pdx1*^{OE} (Fig. 2I). The $YFP^{+}Pdx1^{HI}$ α cells showed diminished MafB but maintained Arx (Supplemental Fig. 8), suggesting a substantial barrier against full-scale suppression of the intrinsic α-cell identity. Failure to repress Arx in the great majority of α cells (as in the few residual $Arx^{+}Ins^{+}$ cells in *Neurog3*^{Cre}-*Pdx1*^{OE} adults) (Fig. 3D) could be a limiting factor for successful conversion. Our findings indicate qualitatively discrete competencies

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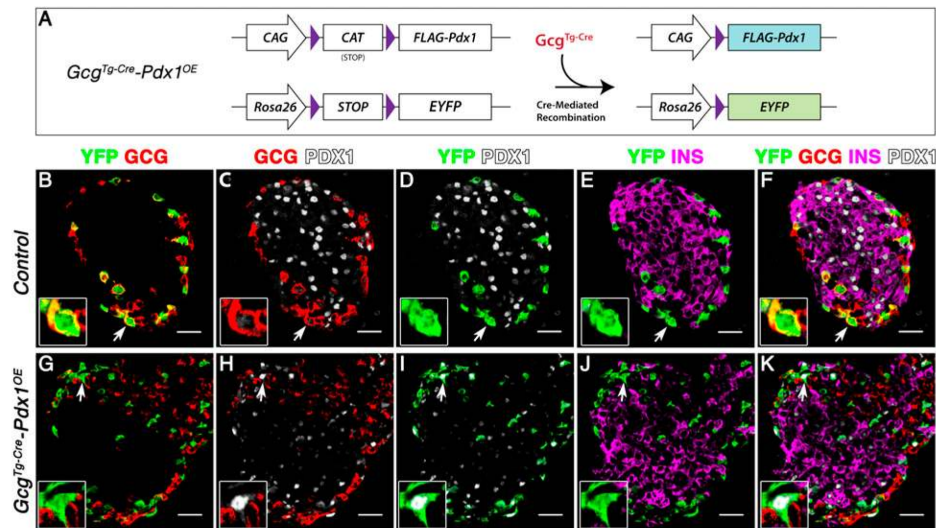

Figure 4. Exogenous *Pdx1* in Gcg^+ cells does not lead to α-cell reprogramming. (*A*) Schematic presentation of *CAG-CAT-Pdx1* and $R26R^{EYFP}$ alleles before and after recombination. (*B–K*) Arrows denote representative cells in *insets* shown with separated channels. Lineage-labeled YFP^+ cells were restricted to mantle Gcg^+ cells (*B*), and ectopic *Pdx1* was only detected in Gcg^{Cre}-$Pdx1^{OE}$ but not in the control among YFP^+ lineage-labeled cells (*D*,*I*). (*K*) Gcg expression was repressed in most YFP^+ cells in Gcg^{Cre}-$Pdx1^{OE}$, but Ins expression was not detected.

between $Neurog3^+$ endocrine progenitor cells and nascent Gcg^+ α cells with respect to their plasticity toward *Pdx1*-enforced reprogramming. The features of lineage-labeled α cells in Gcg^{Cre}-$Pdx1^{OE}$ by molecular marker analysis overlap those of $Neurog3^{Cre}$-$Pdx1^{OE}$ α cells, except that the latter move forward and convert to a β-cell program. No difference in Sst and Ppy expression between Gcg^{Cre}-$Pdx1^{OE}$ and control animals was detected (Supplemental Fig. 9).

We found an even greater resistance to reprogramming of adult α cells when we used a doxycycline-inducible genetic recombination system (Thorel et al. 2010) to delay the time of *Pdx1* activation within Gcg^+ cells until 2–3.5 mo of age (see Supplemental Fig. 10 for details). Most of the lineage-labeled adult α cells (95% of YFP^+Pdx1^{HI} cells) had lost Gcg expression, and <1% showed evidence of Ins expression (i.e., $YFP^+Pdx1^{HI}Gcg^-Ins^-$) (Supplemental Fig. 10). These data clearly show that exogenous *Pdx1* is insufficient to reprogram mature α cells, even over a longer time frame (4–6 wk) than the observed postnatal conversion (~2 wk) effected by expressing *Pdx1* from the $Neurog3^+$ stage onward.

Overall, our findings demonstrate (1) a context-dependent differential competence of *Pdx1* in directing endocrine fate allocation and differentiation and (2) a delayed peri/postnatal response to the early *Neurog3*-based imposition of a cell-autonomous reprogramming stimulus. With respect to affecting the embryonic α- versus β-lineage-commitment process, exogenous *Pdx1* expression within the $Neurog3^+$ cell population might not occur early enough in all progenitors. With this reasoning, the cells that move forward initially as "unaffected" α cells (i.e., $Arx^+Gcg^+Pdx1^{HI}Ins^-$) then experience an unknown peri/postnatal trigger to initiate a rapid yet progressive α-to-β conversion. Delaying the activation of exogenous *Pdx1* until the Gcg^+ state, either embryonic or adult, failed to induce any reprogramming, effectively producing an "unprogrammed" state with loss of *Gcg*/*MafB* but not suppression of *Arx* or gain of *Ins*. The differential response strongly suggests a requirement for *Pdx1*-mediated early competence priming in order to exert future cell conversion, and further, that reprogramming might be separable into modes of either unprogramming or a more profound switching of cell fate.

The unexpectedly delayed postnatal α-to-β conversion leads to speculation on the mechanism of this temporally cryptic priming, which could be referred to as a "chromotypic effect"—being encoded at the chromatin level, without necessarily causing an immediate explicit phenotypic alteration. Learning how *Pdx1* pioneers chromatin priming or epigenetic landscaping could be important with respect to cell reprogramming in vitro and in vivo. It is pertinent to note that *Pdx1* has been implicated in epigenetic modification via interaction with a pancreatic islet-enriched histone methyltransferase, Set7/9, a *Pdx1*-responsive factor proposed to enhance chromatin accessibility and transcription of β-cell genes (Deering et al. 2009; Ogihara et al. 2009). A switch of Set7/9 subcellular localization from cytoplasmic/nuclear (α cell) to exclusively nuclear (β cell) was observed in adult $Pdx1^{HI}$ α cells in our various genetic conditions (Supplemental Fig. 11). This finding suggests that exogenous Pdx1 begins to initiate an epigenetic reconfiguration from α to β, potentially influencing the recruitment of Set7/9 to certain β-cell-specific loci. We also note that, despite *Pdx1*-driven Set7/9 nuclear entry, the *Gcg*-mediated $Pdx1^{OE}$ α cells (embryonic and adult) remain refractory to reprogramming (Fig. 4; Supplemental Fig. 10). Identifying ways to switch certain forms of epigenetic coding associated with this resistance, perhaps determined via flow cytometry-based cell capture, could improve reprogramming efficiency.

The peri/postnatal conversion of $Neurog3^{Cre}$-$Pdx1^{OE}$ α cells raises the possibility that part of the final process of normal islet cell development involves cells checking their "internal transcriptional status," with considerable pathway shifting still possible if the epigenetic state is not completely fixed toward specific fates. The nature of any associated peri/postnatal developmental cue is unknown, and could be organ-local, systemic, or presumably

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even metabolic. How these ideas relate to MafA/MafB resolution (the change from common production of both factors in immature β cells toward the mature pattern of $MafA^+$ cells or $MafB^+$ α cells), which also occurs perinatally (Artner et al. 2010), is a potentially fruitful area for future study. Determining the nature of regulatory checkpoints in the final maturation of endocrine cells could be germane to β-cell differentiation in vitro. The genetic models here could also provide a novel platform for identifying and manipulating cellular maturation and understanding how plasticity could be induced or restricted. Such principles might be applicable to multiple cell types in the pancreas and, more generally, to other organ systems.

Materials and methods

Mice

Information on mouse strains is in the Supplemental Material. All animals and embryos used were PCR-genotyped. Animal handling was under protocols approved by Vanderbilt University Medical Center Institutional Animal Care and Use Committee, or according to the Direction Générale de la Santé of the Canton de Genève.

Immunohistochemistry and morphometric analysis

Tissues were prepared as described (Fujitani et al. 2006). Information on antibodies and morphometric methods are in the Supplemental Material. Statistical analysis was performed using single-factor ANOVA tests and significance determined by $P < 0.05$.

qRT–PCR

RNA isolation (Trizol, Invitrogen), DNase treatment (Ambion), cDNA synthesis, and qPCR (SYBR green, Bio-Rad) were performed. Three samples per genotype per stage were collected, and qPCR was performed at least twice on each sample to determine ΔC_T. Results were shown as $\Delta C_T \pm$ SEM and subjected to Student's *t*-test to determine significance ($P < 0.05$).

Acknowledgments

We thank A. Leiter for *Neurog3*$^{TgBAC\text{-}Cre}$; T. Miyatsuka and H. Kaneto for *CAG-CAT-Pdx1* mice; and P. Collombat, G. Gu, and B. Sosa-Pineda for Arx, Neurog3, and Pax4 antibodies. We also thank R. Stein, G. Gu, M. Gannon, and members of Wright laboratory for discussions. The work was supported by JDRF to Y.P.Y. and NIH U19 DK 042502 and U01 DK 089570 to C.V.E.W.

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Genes Dev. 2011, **25:**
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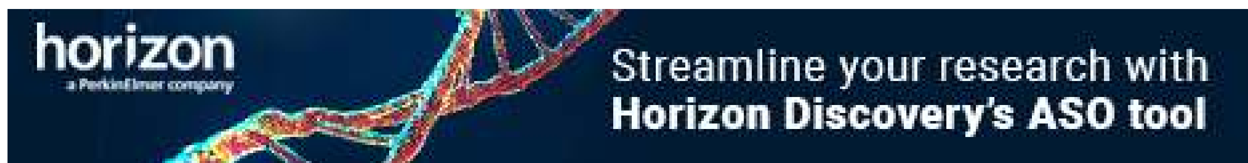

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