Area IV Knockout Reveals How Pdx1 Is Regulated in Postnatal β-Cell Development

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Pdx1 has long been suspected to be a master transcriptional regulator with essential roles in pancreatic development and β-cells (1–5), but the upstream mechanisms that determine Pdx1 gene expression in mature β-cells are poorly understood. In embryonic pancreatic development, Pdx1 is required to initiate branching morphogenesis and early islet formation (6,7). Pdx1 also has pivotal postnatal roles in mature β-cell function and expansion (8,9). Several conserved 5′ *cis*-regulatory regions (areas I–III) influence Pdx1 gene expression in embryonic development (Fig. 1) (10,11). In contrast, another conserved region (area IV) has been implicated in postnatal β-cell maturation (12). However, the master themes that govern Pdx1 gene expression remain a mystery, as cell lines and global knockouts cannot deconvolute how Pdx1 is dynamically regulated in embryonic and postnatal life.

Enter Stein, Wright, and colleagues, who have generated several mouse models to precisely interrogate the Pdx1 promoter (5, 10–16). In embryonic life, Pdx1 gene disruption results in pancreatic agenesis, exocrine insufficiency, and neonatal diabetes, thus revealing the essential role of Pdx1 in early pancreas development (6). This embryonic phenotype is mostly recapitulated by combined deletion of areas I, II, and III (11). Subsequent studies specified areas I and II as regulators of Pdx1 expression in β-cell development, predominated by area II and potentiated by area I (10,13–15). area III may also influence Pdx1 in embryogenesis (16). Notably, area III contains a binding site for Ptf1a, key for pancreas and acinar development (5,16).

Regulation of Pdx1 gene expression in postnatal life is complex and less well understood. Several major elements of the Pdx1 promoter may influence postnatal β-cells. While area I may have a role in mature β-cells to potentiate area II activity (14), area II may also be required for β-cell lineage commitment and maturation (15). Deletion of area II with loss of one Pdx1 allele ($Pdx1^{\Delta AII/-}$) results in mice that are severely hyperglycemic with neonatal lethality. Area II mutant mice contain cells coexpressing insulin and glucagon after birth (15), consistent with reports that Pdx1 maintains β-cell identity in part by repressing α-cell genes (17). Similarly, area II compound deletions restricted to endocrine progenitors ($Ngn3^{Cre}$) or β-cells (RIP^{Cre}) lead to elevated α-cell markers and reduced β-cell factors. Meanwhile, the postnatal role of area III is unclear, as Pdx1 becomes restricted to β- and δ-cells in late development (5).

In this issue of *Diabetes*, Spaeth et al. (18) shift focus to area IV, uncovering the unique and powerful impact of area IV upon postnatal β-cells. In contrast to previously described area I–III mutations, area IV–specific knockouts ($Pdx1^{\Delta AIV/\Delta AIV}$) did not induce developmental or early postnatal β-cell defects. To further interrogate area IV, mice with deletion of area IV were combined with loss of one Pdx1 allele ($Pdx1^{\Delta AIV/-}$). These area IV mutants exhibited reduced $Ngn3^{+}$ progenitors and differentiated insulin^{+} and somatostatin^{+} cells (18), a milder phenotype than area II mutants (15). The primary effects of area IV were observed in postnatal life after weaning. Male area IV mutant ($Pdx1^{\Delta AIV/-}$) mice had reduced Pdx1 at 5 weeks of age and defective β-cell function, with reduced expression of β-cell–defining genes such as insulin, MafA, Nkx6.1, and Glut2. Notably, reduced Pdx1 expression in area IV mutants was sufficient to suppress α-cell lineage genes (18), contrary to previous reports (15). As expected, β-cell proliferation and area were substantially reduced in male area IV mutants. These studies reveal that area IV regulates Pdx1 control of postnatal β-cell function and growth.

The early postweaning period appears to be a novel window for Pdx1 regulation of β-cells. Using wild-type islets, Spaeth et al. (18) demonstrated increased Pdx1 binding to area IV by chromatin immunoprecipitation analysis, perfectly concordant with the reduction in β-cell proliferation and functional genes. The authors strategically annotated published data sets describing Pdx1 DNA binding sites from mouse βTC-6 cells, with differentially expressed genes during weaning. Their alignment revealed Pdx1 binding sites within one-third of differentially expressed genes from weaned islets. Subsequent assessment of area IV mutant islets demonstrated

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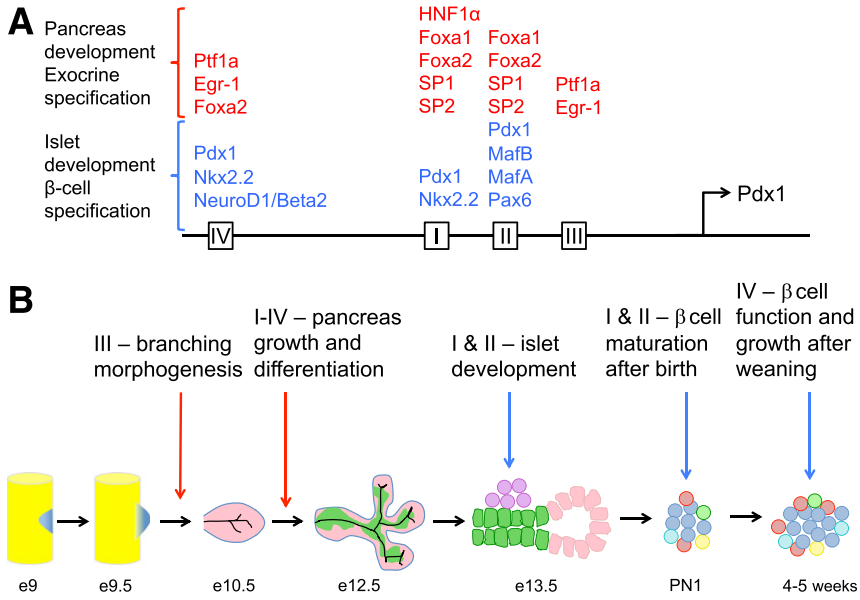

Figure 1—Enhancer region areas I–IV of the Pdx1 promoter and the regulation of Pdx1 in pancreas development and postnatal β-cells. *A*: A depiction of the conserved 5′ *cis*-regulatory regions (areas I–IV) upstream of the Pdx1 transcription start site. Transcription factors with known binding sites within each area are described for those with roles in pancreas development and exocrine specification (red) and others in islet development and β-cell specification (blue). *B*: Each enhancer element (areas I–IV) is mapped to its embryonic or postnatal function. Briefly, pancreas specification occurs around embryonic day (e) 9, which leads to budding of the dorsal and ventral pancreas, initiating the "primary transition" (5). Area III control of Pdx1 is required for subsequent branching morphogenesis on e10.5, with some possibly overlapping roles for area I and II in epithelial growth of the pancreas through to e12.5 (6,11,16). Area I and II play important roles in Pdx1 specification of $Ngn3^+$ islet endocrine progenitors beginning at ~e13.5, initiating the secondary transition (5,15). Area I and II also regulate Pdx1 control of β-cell maturation and repression of α-cell lineage after birth (postnatal day 1 [PN1]) (15). Lastly, Pdx1 regulation by area IV has a novel role after the onset of weaning (4–5 weeks of age) to mediate β-cell function and growth (18).

several Pdx1-bound genes involved in replication and oxidative phosphorylation were decreased. Together, this suggests a potentiation effect of Pdx1 during a critical window for β-cell function and proliferation.

Collectively, these studies provide novel insights into Pdx1 regulation through distinct regional and temporal mechanisms. Absent or reduced Pdx1 induces neonatal diabetes and maturity-onset diabetes of the young and contributes to type 2 diabetes (5). Therapeutic strategies to increase Pdx1 through targeting of area II might benefit developmental and maturation defects associated with maturity-onset diabetes of the young and neonatal diabetes, while targeting area IV might improve mature β-cell function and growth in type 2 diabetes. Indeed, small molecules have been identified that increase Pdx1 expression (19); however, the specific regulatory region activated might dictate Pdx1 function based on growing evidence from Stein, Wright, and colleagues. Notably, these observations also have important consequences for directed differentiation of stem/pluripotent cells into endocrine progenitors and subsequently for β-cell maturation, function, and expansion.

Area IV mice exhibit sexual dimorphic phenotypes, indicating that some intricacies of Pdx1 gene regulation remain unresolved. Female area IV mutant mice were phenotypically identical to controls, in contrast to male mutants. Further investigation into the sex-specific effects of area IV will be important, as it could reveal important biology regarding the impact of sex steroids upon Pdx1 gene regulation and diabetes phenotypes (20). However, the effects of sex steroids upon Pdx1 regulation might occur through nonclassic mechanisms, as Spaeth et al. (18) did not find typical androgen or estrogen response elements within areas I–IV.

In conclusion, the interrogation of area IV regulation of Pdx1 by Spaeth et al. (18) provides a full molecular elucidation of the roles of Pdx1 played out in vivo. These studies expand the existing literature to reveal unique transcriptional regulatory and subsequent functional roles of Pdx1 in embryonic and postnatal life (Fig. 1). Area IV has distinct roles in Pdx1 expression upon postnatal β-cell function and growth during a novel period after weaning. Future studies on the upstream regulation of Pdx1 will have great value to unravel the mysteries of β-cell development, function, and adaptation to diabetes.

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