subtype ratios as seen in *Nkx2.2*$^{-/-}$ mice, suggesting a function for *Nkx6.1* downstream of *Nkx2.2* (93). Nkx6.1 is broadly expressed in the pancreatic epithelium from e9.0, is later expressed in endocrine progenitors, then subsequently becomes β-cell–restricted (93). Unlike *Nkx2.2* mutants, *Nkx6.1*-null mice specifically lose only the β-cell lineage (93). The β-cell loss in *Nkx6.1*-deficient mice could, however, also be secondary to the early endocrine to acinar cell fate switch caused by *Nkx6.1* abrogation in MPCs (62), thus illustrating the general limitation of studying specific gene functions in null mutant animals. The specific role of Nkx6.1 in β-cell specification/differentiation remains to be defined and will require conditional gene inactivation in a temporally and spatially regulated manner.

The recent dissection of the role played by Arx in endocrine cell development has yielded insight into the mechanisms regulating the segregation of the α- and β-cell lineages. Arx is expressed in a Ngn3-dependent fashion from e9.5 in the pancreas whereafter its expression becomes confined to α- and PP-cells (94,95). Abrogation of *Arx* activity causes α-cell deficiency while numbers of β- and δ-cells are increased (94), revealing Arx to be both required for α-cell fate acquisition and repression of β- and δ-cell fate, the mirror opposite of the role of Pax4 with which Arx is coexpressed during early endocrine differentiation. Concordantly, ectopic *Arx* expression in the endocrine precursors or mature β-cells expands α- and PP-cell numbers at the expense of β-cells (95), showing Arx to be both necessary and sufficient to promote α- and PP-lineage commitment. These findings are consistent with studies showing that Pax4 and Arx mutually and directly inhibit one another's transcription at the promoter level (96). The observation that both α- and β-cells in *Pax4; Arx* compound mutant mice are replaced by δ-cells, which later initiate ectopic PP expression (96), has led to a refinement of the model by which Pax4/Arx govern islet cell subtype choices downstream of Ngn3. Namely, it has been proposed that when one of the two factors is activated in preference to the other in endocrine progenitors, Arx specifies α-cell fate while Pax4 first permits commitment toward a β-/δ-cell fate by repressing *Arx* then subsequently driving a hypothetical bipotential β-/δ-cell precursor cell toward a β-cell fate (96) (Fig. 3 Model A). This prevailing model is based on the assumption that two cells with different endocrine subtype identity arise from an intermediary progenitor downstream of Ngn3, which is in apparent contradiction to the recent observation that each Ngn3^{+} cell only gives rise to one endocrine cell and is therefore unipotent (77). Reconciling this apparent contradiction, an alternative explanation for the observed phenotypes is that Arx and Pax4 function to stabilize lineage decisions downstream of Ngn3 rather than reallocating cells at the level of a hypothetical intermediate precursor cell (Fig. 3 Model B). Additional studies are clearly required to determine when and how endocrine subtype identity is established and stabilized during development.

Similar to Arx and Pax4, most recently, loss-of-function studies in both mouse (97) and zebrafish (98) have intriguingly hinted at a role for the transcription factor Rfx6 in controlling endocrine cell subtype choice. Rfx6, which is activated by Ngn3, is expressed broadly throughout the epithelium of the mouse gut by e9.0 and within a subset of endocrine progenitor cells during the secondary transition, becoming restricted to islet cells by adulthood (97,98). *Rfx6* exhibits a parallel expression pattern in zebrafish pancreas (98). Consistent with its endocrine expression, mice lacking *Rfx6* exhibit a loss of all mature endocrine cell types with the notable exception of the PP-cells, which unusually express the β-cell marker Nkx6.1 (97). In zebrafish, *Rfx6* abrogation results in failure of the non–β-cell endocrine lineages to differentiate from endocrine progenitors while the β-cells fail to coalesce into an islet (98). Future studies will be needed to determine the specific roles played by Rfx6 in the transcription factor network orchestrating endocrine differentiation.

ISLET FORMATION AND β-CELL MATURATION

As birth nears, the exocrine pancreas grows rapidly, primarily through mitotic expansion of differentiated acinar cells. Meanwhile, from e16.5 onwards, the endocrine cells coalesce into polyclonal clusters to form functional islets that, in the mouse, comprise a central core of β-cells and an outer mantle composed of the other four endocrine cell types. Disrupted islet cytoarchitecture following β-cell–specific deletion of cadherins or neural cell adhesion molecule has revealed a requirement for cell adhesion molecules in β-cell–β-cell adhesion during islet formation (99,100). In addition, in vitro evidence (101) supports a role for matrix metalloproteinase enzymes in facilitating endocrine cell migration via extracellular matrix degradation. However, this hypothesis has not been borne out in vivo (102).

During the early postnatal period, the β-cells acquire the ability to regulate insulin secretion in response to glucose (103), requiring the expression of the glucose transporter Glut2 and prohormone convertase PC1/3 to cleave proinsulin to active insulin. The recent characterization of the roles played by the transcription factors MafA and MafB in pancreas development has offered insight into the mechanisms governing β-cell terminal differentiation. MafA, which was initially identified as a β-cell–specific activator of insulin transcription (reviewed in [104]), is exclusively expressed in insulin^{+} cells from e13.5 onward (105). In contrast, MafB is expressed in both insulin^{+} and glucagon^{+} cells by e12.5 but becomes restricted postnatally to α-cells (106). Thus, maturing β-cells undergo a developmental MafB→MafA switch (107). Apparently conflicting with its expression in immature insulin^{+} cells, MafA function is dispensable for β-cell development, most likely because of functional compensation by MafB (108). However, deletion of *MafB* reduces numbers of insulin^{+} and glucagon^{+} cells and delays the development of insulin^{+} cells until the onset of *MafA* expression (109). Loss of *MafB* is associated with downregulation of factors required for β-cell maturation and function such as Pdx1, MafA, Nkx6.1, and Glut2. MafB is thus crucially required for the terminal differentiation of both α- and β-cells by acting as a master activator of hormone gene transcription and key regulators of β-cell differentiation and function.

Serving as a potent example of individual transcription factors exhibiting multiple roles during pancreatic development, conditional deletion of *Isl1* has recently unmasked a crucial requirement for this transcription factor in endocrine cell maturation (110). Ablation of *Isl1* immediately prior to the secondary transition results in a severe reduction in the number of mature endocrine cells prior to the eventual loss of endocrine cell mass contributed by the changes in cell proliferation and survival (110). Paralleling the multiple roles played by Isl1 in pancreatic organogenesis, recent studies have also uncovered

additional, later roles for Ngn3 and its downstream target, NeuroD1, in the acquisition and maintenance of the terminally differentiated, fully functional β-cell phenotype (75,111). Future work will undoubtedly focus on further elucidating the roles of Isl1 and other endocrine differentiation factors in β-cell maintenance and function as the requisite genetic tools become available.

FUTURE PERSPECTIVES

Despite the wealth of knowledge we have amassed to date, many questions in the field of pancreas and β-cell development remain unanswered, as this review has sought to illustrate. It is only relatively recently, for example, that evolving transgenic mouse technology has provided insight into the roles of transcription factors such as Ptf1a and Sox17 in allocating pancreatic fate at the expense of other endodermally derived organs. The inability of any single gene deletion to prevent formation of the early pancreatic anlage hints at the complexity of the transcriptional network governing this process and the involvement of as yet unidentified players, which further work will undoubtedly unmask. How known transcriptional regulators interact with one another and with extraneous signaling pathways is also ripe for future examination, whether in the control of pancreatic specification, growth, or cytodifferentiation.

Outstanding among current questions in the field is the issue of multipotency of individual progenitors at single-cell resolution. This area of investigation would greatly benefit from the development of a culture system in which organ development can be initiated from single cells in vitro, as recently established for cells in the intestinal crypts (112). Another still understudied area is the question of whether or how the physical location of cells in the developing pancreas bestows lineage-restriction upon progenitors. Open questions include whether all ductal cells in the secondary transition progenitor cords function as progenitor cells; whether such progenitors are homogeneously or heterogeneously lineage-restricted; and if the latter, the factor(s) governing progenitor commitment or differentiation into ductal, endocrine, or acinar lineages. Finally, as illustrated in this review, we still know very little about when and how the five different endocrine cell subtypes are specified during development. It is anticipated that much effort will be expended in the future with the goal of answering some of these questions. It is hoped that acquiring more comprehensive insight into the processes governing β-cell neogenesis in vivo will enable the in vitro generation of unlimited quantities of functional insulin-producing cells for the successful management and eventual cure of diabetes.

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