The Hypothalamus and β-Cell Connection in the Gene-Targeting Era

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After years of debate, Le Douarin's elegant work (1) established that pancreatic islet cells differentiate from progenitors emerging from the definitive gut endoderm rather than from neuroectoderm as had been inferred from co-expression of neuronal markers. Although islet cells and the brain do not share a common developmental origin, a fascinating picture has emerged in which they nonetheless share many biochemical pathways and, hence, are characterized by extensive overlap in gene expression. Brain and islet are also tightly linked functionally through neural-entero-islet, brain-islet, and islet-brain axes (2,3). Thus, the secretion of insulin and other islet hormones are clearly regulated by the hypothalamus and other brain areas, while conversely insulin action in the hypothalamus influences both energy balance (4) and glucose metabolism (5). Not surprisingly, therefore, targeted deletion or induction of genes in either tissue can yield mice with overlapping phenotypes where hormone secretion and glucose metabolism are concerned. The report by Wicksteed et al. (6) in this issue of *Diabetes* sheds welcome light on the extent to which commonly used mouse models for β-cell–specific gene targeting affect gene expression in the brain as well as in the islet.

A commonly employed strategy for gene targeting in vivo employs the *Cre/LoxP* system of DNA recombination that allows for either deletion or de novo induction of select gene-coding sequences in specific cell types in mice (7–9). Tissue specificity with this method is achieved through the use of cell type-specific promoters to drive expression of *Cre* recombinase, an enzyme that cleaves DNA sequences between flanking *LoxP* sites. These promoters can be further modified to incorporate drug-responsive elements, allowing *Cre* recombinase expression to be switched on at will by drugs such as tamoxifen (*CreERT*). The *Cre/LoxP* system has thus emerged as an essential strategy with which to investigate the spatial and temporal function of a given gene (10–13) and has also helped define cell lineage relationships through the induction of reporter genes (14–16).

The capacity to alter islet cell function by directing *Cre* expression to specific cell populations has been widely and productively employed by diabetes investigators (for a complete list of transgenic mice directing expression of *Cre* in pancreatic cell populations, refer to http://www.findmice.org/index.jsp and http://www.informatics.jax.org/). Commonly employed mouse models use either the rat *insulin2* promoter (RIP) or the *Pdx1* promoter to drive Cre recombinase in β-cells. Of three commonly used *RIP-Cre* mouse lines, *Cre* is expressed constitutively in two, while expression is tamoxifen-inducible in the third (17–19). Similarly, Cre expression is constitutive in three of four published *Pdx1-Cre* mouse lines, while it is tamoxifen-inducible in the fourth (20–23).

Because of differences in the timing of Cre-recombinase expression, *Pdx1-Cre* mouse lines have been labeled "early" or "late" recombinants (e.g., *Pdx1-Cre^{Early}* [21]) and *Pdx1-Cre^{Late}* [19]). The difference in timing of recombination is important since in addition to other key variables (e.g., tissue distribution and degree of recombination), the age at which altered gene expression occurs can have a dramatic impact on phenotypic outcomes. This effect is illustrated in a study (24) in which the use of *Cre-LoxP* technology to introduce a stable mutant of β-catenin within the mouse pancreatic epithelium had opposite effects depending on the spatial and temporal pattern of gene induction (25). Thus, when this form of β-catenin was expressed during early organogenesis using *Pdx1-Cre^{Early}* mice (21), a severe reduction of pancreas mass associated with postnatal lethality was observed (due to the loss of Pdx1 expression in early pancreatic progenitors). By comparison, induction at a later time point using *Pdx1-Cre^{Late}* mice (19) increased cellular proliferation and induced a dramatic increase of pancreas organ size (24). Should Cre recombinase be expressed in other tissues, therefore, the phenotypic consequence may also depend on the age at which recombination occurs.

In this issue of *Diabetes*, Wicksteed et al. (6) compared *Cre* activity in the brain of commonly used mouse models with that observed using a new mouse line generated using a tamoxifen-inducible mouse *insulin1* promoter to drive *Cre* expression (*MIP-Cre/ERT*). The investigators report that each of the three commonly used *RIP-Cre* transgenic lines exhibit Cre-mediated recombination in the brain (Fig. 1). In one of these mouse lines (*RIP-Cre^{Mgn}*) (18) (Fig. 1*B*), 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) staining was detected throughout the brain with the highest intensity in the midbrain and ventral regions, whereas *RIP-Cre^{Herr}* mice displayed a weaker and more punctate central nervous system expression pattern without obvious regionalization (19) (Fig. 1*C*). The tamoxifen-inducible *RIP-Cre/ERT* mouse (17) also displayed strong, punctate X-gal staining in the brain but with a more restricted expression pattern (Fig. 1*D*). Analysis of *Pdx1-Cre* lines also revealed X-gal staining in the brain, including one (*Pdx1-Cre^{Early}*) (21) (Fig. 1*E*) in which Cre activity was detected in distinct hypothalamic neuronal subsets impor-

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DOI: 10.2337/db10-1149
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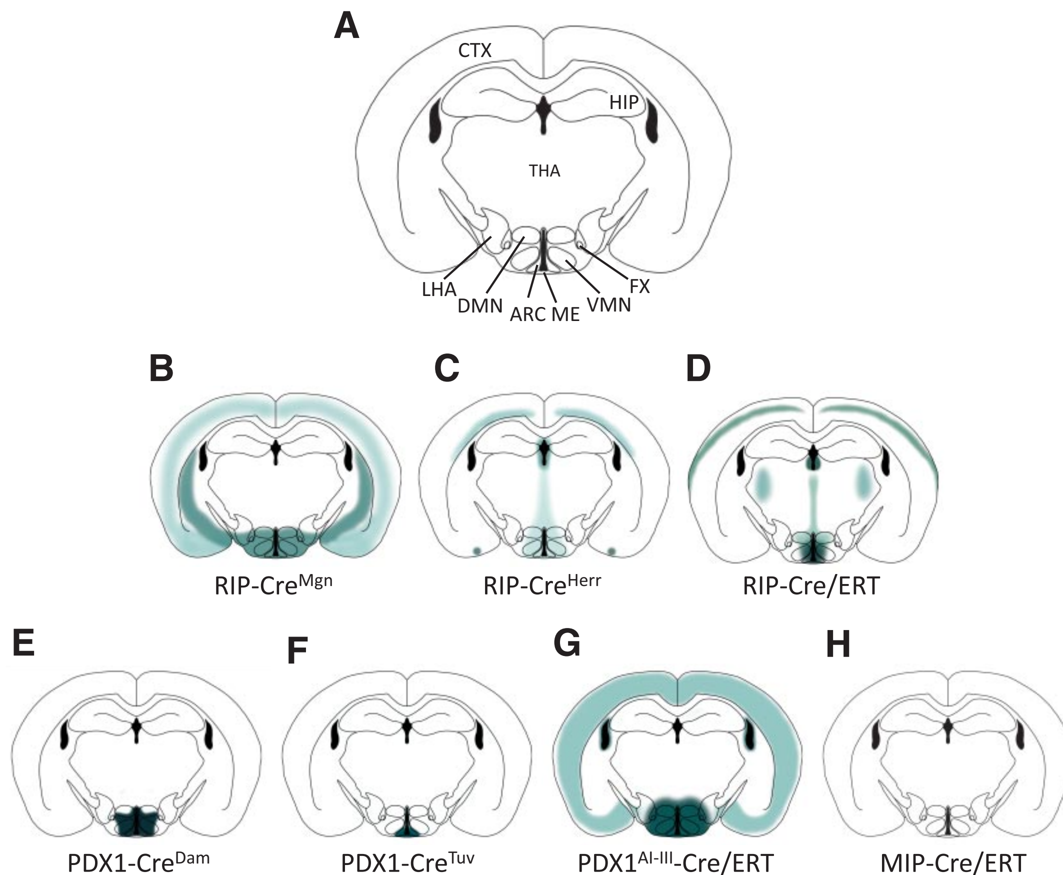

FIG. 1. Schematic representation of Cre expression in a coronal section taken through the mid-hypothalamus of mice with pancreas-targeted Cre drivers that were crossed with the *R26R β-galactosidase Cre* reporter line. Shaded areas depict regions of Cre-mediated recombination based subjectively on findings reported by Wicksteed et al. (6). *A*: *R26R$^{wt/lacZ}$* without a *Cre* driver. *B*: *RIP-Cre^{Mgn}*; *R26R$^{wt/lacZ}$*. *C*: *RIP-Cre^{Herr}*; *R26R$^{wt/lacZ}$*. *D*: *RIP-Cre/ERT*; *R26R$^{wt/lacZ}$*. *E*: *PDX1-Cre^{Dam}*; *R26R$^{wt/lacZ}$*. *F*: *PDX1-CreTuv*; *R26R$^{wt/lacZ}$*. *G*: *PDX1^{AI-III}-Cre/ERT*; *R26R$^{wt/lacZ}$*. *H*: *MIP-Cre/ERT*; *R26R$^{wt/lacZ}$*. Until more is known regarding the reproducibility of these expression patterns, this depiction is intended only as a general guide. Investigators using these mice are advised to evaluate central nervous system Cre-mediated recombination on a case-by-case basis. ARC, arcuate nucleus; CTX, cortex; DMN, dorsomedial nucleus; FX, formix; HIP, hippocampus; LHA, lateral hypothalamic area; ME, median area eminence; THA, thalamus; VMN, ventro-medial nucleus.

tant in metabolic regulation, including both orexin-expressing neurons and neurons shown to be activated by leptin. Since expression of genes targeted using these mouse lines will be altered in the brain as well as in the pancreas, these findings offer a cautionary note to the interpretation of resultant phenotypes.

Compared with the above *Cre* models, the new *MIP-Cre/ERT* mice described by Wicksteed et al. display a more β-cell–specific recombination pattern with virtually undetectable Cre activity in any brain regions analyzed (Fig. 1*H*). As this mouse model also features a tamoxifen-inducible Cre, it offers the potential for both temporal and tissue-specific control of gene deletion (or induction) in pancreatic β-cells and, hence, is a welcome addition to the repertoire of animal models used by the diabetes research community. Whether efficient *Cre*-mediated recombination in *MIP-Cre/ERT* mice can be achieved in utero and therefore can be used to target select genes during β-cell development is a question that awaits further study.

Although somewhat tangential to the question at hand, it seems perplexing that the rat *insulin2* promoter, but not the mouse *insulin1* promoter, is active in the mouse brain. Several factors likely contribute to this discrepancy, including the additional regulatory elements within the larger promoter fragment employed in *MIP-Cre/ERT* mice, but an important conclusion supported by this finding is that the insulin gene does not appear to be expressed in adult mammalian brain. This finding adds to a literature that strongly supports this conclusion despite papers that appear from time to time implying the opposite (most recently in the Alzheimer disease literature) (26).

It seems likely that the question of whether altered neuronal gene expression influenced the phenotype of various published *RIP-Cre* or *Pdx1-Cre* mouse models will soon be answered. In the meantime, however, *RIP-Cre* and *Pdx1-Cre* models will continue to be important tools for the study of islet development and function as long as a neural contribution to a particular phenotype can be reliably excluded, and improvements in this technology will undoubtedly continue. As this progress unfolds, we are reminded that the close functional link between brain and islet can be a source of confusion and frustration, as well as one of fascination.

ACKNOWLEDGMENTS

No potential conflicts of interest relevant to this article were reported.

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