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## Human Growth Hormone in Transgenesis: A Growing Problem?



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Temporospatial overexpression or inactivation of genes in mouse models has undeniably boosted biomedical research. This is most commonly achieved using the Cre-loxP technique, where mice expressing Cre recombinase driven by a tissue-specific or inducible promoter are bred with mice in which one or more exons of a gene of interest are flanked by LoxP sites (1). This allows for a more in-depth view of the gene function in the context of a particular time frame and tissue system, while avoiding unwanted effects due to the absence of this gene during development.

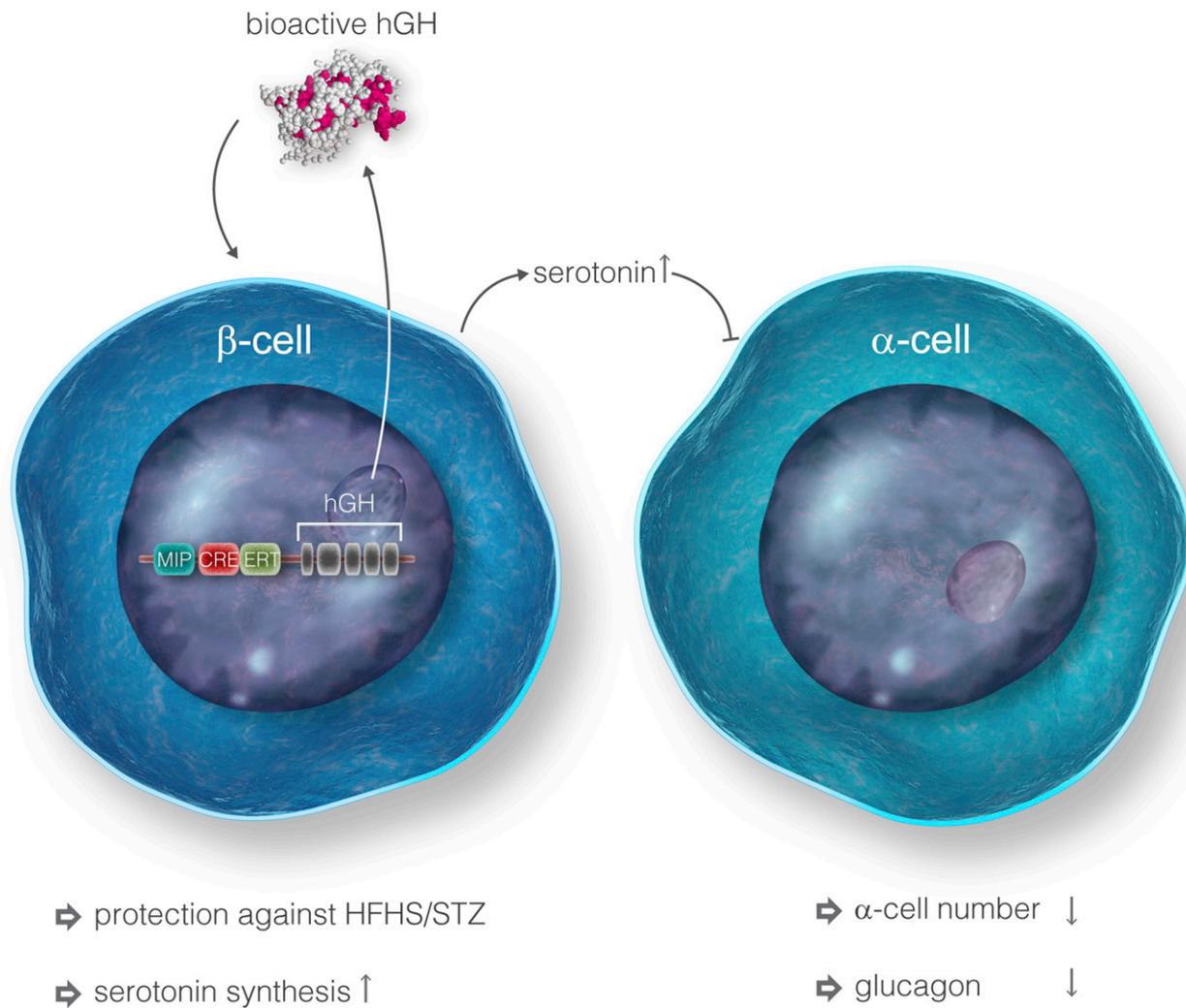
However, in the recent years several pitfalls have emerged related to this technique (2–4). There have been reports of disturbed endogenous gene expression at the site of the Cre-transgene insertion (4), low or mosaic Cre expression (5,6), and expression in undesired tissues, such as unwanted Cre activity in nutrient-sensing neurons from transgenic mice that express Cre under the control of *Ins2* and *Pdx1* promoters (7,8). Equally disturbing is the finding that high Cre activity can cause chromosomal rearrangements, apoptosis, and decreased proliferation (9–11). Particularly for diabetes and central nervous system research, these caveats have been reviewed in detail (2,3).

In this issue of *Diabetes*, Oropeza et al. (12) describe yet another concern in the tamoxifen-inducible MIP-CreERT<sup>1Lphi</sup> mouse line caused by transgenic human growth hormone (hGH) expression. In the 1980s, a pioneering study described that the presence of introns induces more efficient transgene expression (13). Soon after, this principle was demonstrated using heterologous introns in animals (14). In the early 1990s, the entire *hGH* gene was inserted downstream of the Cre fragment as a transgene enhancer (15) and has become a routine strategy ever since. Recently, however, it has been shown that this can result in a bicistronic Cre-hGH mRNA. As a result, the mRNA is translated not only in Cre recombinase but also in functional hGH (16,17). Interestingly, hGH can activate both the growth hormone receptor and the prolactin receptor (PRLR) (17,18). Particularly on  $\beta$ -cells, hGH acts as a lactogen and binds to the

PRLR present on  $\beta$ -cells, hereby inducing pregnancy-related changes, such as an increase in  $\beta$ -cell mass and insulin content, induction of serotonin synthesis, and protection against the  $\beta$ -cell toxin streptozotocin (STZ). Next to a pregnancy phenotype, impaired glucose-stimulated insulin secretion and decreased  $\beta$ -cell GLUT2 levels were observed.

The MIP-CreERT<sup>1Lphi</sup> mouse line was welcomed in the field as it was the first that used an 8.5-kb fragment of the mouse *Ins1* promoter, driving Cre activity exclusively to  $\beta$ -cells while being absent in the brain (7). In addition, no adverse effects on glucose tolerance and glucose-stimulated Ca<sup>2+</sup> release were observed (19). However, Oropeza et al. (12) provide a more extensive and detailed analysis of this mouse model. First, strong evidence is provided using higher resolution techniques and the mT/mG reporter line (20) that Cre activity is indeed exclusively found in  $\beta$ -cells and not in hypothalamic or hindbrain nuclei, as was reported before in other strains (7,8). Furthermore, whole-body glucose homeostasis was similar to controls, both on normal chow and after a high-fat, high-sucrose (HFHS) diet. However, they observed that despite secreting normal amounts of insulin upon in vitro glucose stimulation there was an increase in pancreatic islet number and insulin content. Strikingly, MIP-CreERT animals on both C57BL/6 and FVB backgrounds were strongly protected against the type 2 diabetes-inducing HFHS/STZ regimen. Significant resistance was also seen for single high-dose STZ administration. Similar to the previously reported strains (17), MIP-CreERT mice express high levels of hGH in islets, independent of tamoxifen administration. After autocrine/paracrine activation of the PRLR, this results in *Tryptophan hydroxylase-1* (*Tph1*) and *-2* (*Tph2*) expression, which in turn induces serotonin biosynthesis. Finally, the authors provide evidence that serotonin negatively affects compensatory  $\alpha$ -cell proliferation and glucagon secretion upon HFHS/STZ in this mouse line (Fig. 1).

Interestingly, Oropeza et al. (12) did not observe impaired glucose-stimulated insulin secretion and decreased GLUT2 levels as was shown before in the *Pdx1-Cre*<sup>Late</sup>



**Figure 1**—MIP-CreERT mice express high levels of bioactive hGH in pancreatic  $\beta$ -cells. This activates the prolactin receptor, which induces serotonin biosynthesis and causes protection against HFHS/STZ.  $\beta$ -Cell serotonin has a paracrine effect on pancreatic  $\alpha$ -cells, negatively influencing  $\alpha$ -cell number and glucagon secretion (12).

model that also expresses hGH in islets (17). This suggests that these impairments are independent of hGH bioactivity. Another explanation could be a gene-dose response of hGH in different lines, as the levels of hGH protein could depend on the promoter type, insertion site, and the mouse strain, as suggested previously (21). Adding quantification of absolute islet hGH levels by ELISA or high-performance liquid chromatography would allow a direct comparison of hGH levels between mouse strains. Next to islets, lower hGH mRNA levels were also detected in the arcuate nucleus and ventromedial hypothalamus, but the question remains as to whether this has considerable effects on physiology. For example, whole-genome expression profiling on these microdissected hypothalamic regions could possibly address this issue. Finally, this study is the first to demonstrate additional transgene-related effects on pancreatic  $\alpha$ -cells. Whether this phenomenon also occurs in other pancreatic driver lines expressing hGH could be the subject of future research.

Reports such as this are crucial to help move the field forward. The hGH minigene has been used as a transgene enhancer since the beginning of conditional mouse targeting, extending into various research areas, with a total number of lines exceeding well over 200 (G. de Faudeur, B. Ramos-Molina, B.B., F. Schuit, J.W.M.C., unpublished data). In several lines, bioactive hGH has now been demonstrated in islets, the pituitary, and the hypothalamus. On the other hand, no hGH protein was found in the liver, despite high levels of Cre-hGH mRNA (16). The reason for this tissue-dependent translation is unknown. Validating each line in great detail, such as the MIP-CreERT line, is therefore key to the design of future experiments as well as for the careful reinterpretation of published work.

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

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