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Endogenous beta-galactosidase expression in murine pancreatic islets

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To the Editor:

The tissue-/cell-specific recombinase system, the Cre/loxP system, has become a very useful tool to conditionally inactivate or activate gene function [1–6]. This system is based on the ability of Cre to catalyse the excision of DNA flanked by loxP site recognition sequences. A number of studies on cell lineage in pancreatic cells have been performed by intercrossing cell-specific Cre-expressing transgenic mice and ROSA β geo26 (ROSA26) mice, which carry the gene for bacterial beta-galactosidase (*lacZ*) [7]. In the double transgenic offspring, *lacZ* is expressed where Cre was expressed at a high enough level to excise the floxed stop codon. Commonly, X-gal staining is used to detect beta-galactosidase activity: beta-galactosidase catalyses the hydrolysis of X-gal to an indolyl alcohol, which then oxidises to form an intense blue indigo stain. While specific induction of *lacZ* by precise control of Cre, as well as leakage of beta-galactosidase by spontaneous Cre excision in double transgenic mouse, have been well studied, little work has dealt with the contribution of endogenous beta-galactosidase activity to false-positive results. Heart, liver, spleen, kidney, brain and skeletal muscle from mouse, rat and rabbit [8], and cultured human foetal pancreas [9], have been studied for endogenous beta-galactosidase activity [8]. In cultured human foetal pancreas, the mRNA expression and enzyme activity of beta-galactosidase are developmentally regulated, peaking at 24 weeks of gestation and declining to low levels in the adult [9]. Endogenous beta-galactosidase activity in these organs was rarely detected following incubation at a pH greater than 7.5. In contrast, bacterial beta-galactosidase activity in the ROSA β geo26 was strongly detected at pH

8.0–9.0 [8]. In the mouse pancreas, we evaluated endogenous beta-galactosidase expression and studied factors that may contribute to false-positive results.

Firstly, to determine expression of the endogenous beta-galactosidase mRNA in various tissues, we performed RT-PCR on cDNAs from mouse islets, liver, kidney and spleen as well as from pancreatic beta cells from mouse insulin promoter I (MIP)–green fluorescent protein (GFP) mice [10], purified with FACS by their GFP expression (Fig. 1). The sizes of the PCR products for beta-galactosidase and the internal control TATA-binding protein were 330 and 190 bp, respectively. Pancreatic beta cells and islets as well as other organs expressed endogenous beta-galactosidase (*Glb1*) mRNA (Fig. 1).

Since it is important to determine endogenous beta-galactosidase enzyme activity, we performed X-gal staining on sections of pancreas and kidney from normal adult C57BL/6 mice; endogenous beta-galactosidase is known to be expressed in the kidney. From mice cardiac-perfused

Fig. 1 Expression of the endogenous beta-galactosidase mRNA in tissues. Mouse pancreatic beta cell, islet, liver, kidney and spleen cDNAs were amplified with 35–40 cycles of PCR. Pancreatic beta cells of MIP–GFP mice ($n=12$) were FACS-sorted. Mouse TATA-binding protein (mTBP) was used as a positive control. The size of the PCR products for beta-galactosidase and mTBP are 330 and 190 bp, respectively

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with a fixative solution of 2% glutaraldehyde, 4% paraformaldehyde and 30% sucrose, pancreas and kidney were excised, enrobed in optimal cutting temperature compound and frozen in chilled isopentane. Mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA). All procedures were approved by the Joslin Animal Care Committee in accordance with the 'Guide for the Care and Use of Laboratory Animals'. Bacterial beta-galactosidase has a pH optimum of 6–8, whereas the mammalian enzyme operates at a pH optimum of 3–5; therefore, we examined X-gal staining on tissue sections at two pHs (4.0 and 7.4) over an extended time course. This difference in pH should detect endogenous enzyme activity in mammalian tissues, if there were any.

As shown in Fig. 2, at pH 7.4, pancreatic islets had no enzyme activity with 24 h incubation but had a suggestion of endogenous activity with longer incubations. Neither acinar nor ductal cells were stained at all. In contrast, at pH 4.0, pancreatic islets turned deep blue within 4 h (Fig. 2). Kidney sections (data not shown) studied in parallel showed endogenous beta-galactosidase activity within 24 h even at pH 7.4, and were intensely blue after 4 h at pH 4.0. These results suggest that pancreatic islets express some endogenous beta-galactosidase and that caution is needed in interpreting experiments using localisation of bacterial beta-galactosidase in a few cells within the

Fig. 2 Endogenous beta-galactosidase activity in mouse adult pancreas. The enzyme activity and pattern of expression were determined by X-gal staining at pH 4.0 (e–h) or pH 7.4 (a–d) at the indicated time points (4, 24, 48 and 72 h). Original magnification, $\times 400$

pancreas. Attention must be given to pH during X-gal staining, as well as incubation time, in order to exclude endogenous beta-galactosidase activity.

In conclusion, we found that mouse pancreatic islets, as well as other organs, have endogenous beta-galactosidase. Thus, to obtain reliable results in the pancreas of double transgenic Cre/ROSA β geo26 mice, careful study is required. Care and, as always, proper controls, must be used to have reliable, specific staining of bacterial beta-galactosidase.

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References

1. Rossant J, Nagy A (1995) Genome engineering: the new mouse genetics. *Nat Med* 1:592–594
2. Rossant J, McMahon A (1999) 'Cre'-ating mouse mutants: a meeting review on conditional mouse genetics. *Genes Dev* 13:142–145
3. Nagy A (2000) Cre recombinase: the universal reagent for genome tailoring. *Genesis* 26:99–109
4. Lewandoski M (2001) Conditional control of gene expression in the mouse. *Nat Rev Genet* 2:743–755
5. Kwan KM (2002) Conditional alleles in mice: practical considerations for tissue-specific knockouts. *Genesis* 32:49–62
6. Branda CS, Dymecki SM (2004) Talking about revolution: the impact of site-specific recombinase on genetic analysis in mice. *Dev Cell* 6:7–28
7. Soriano P (1999) Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat Genet* 21:70–71
8. Weiss DJ, Liggitt D, Clark JG (1999) Histochemical discrimination of endogenous mammalian beta-galactosidase activity from that resulting from lac-Z gene expression. *Histochem J* 31:231–236
9. Beattie GM, Levine F, Mally MI et al (1994) Acid β -galactosidase: a developmentally regulated marker of endocrine cell precursors in the human fetal pancreas. *J Clin Endocrinol Metab* 78:1232–1240
10. Hara M, Wang X, Kawamura T et al (2003) Transgenic mice with green fluorescent protein-labeled pancreatic β cells. *Am J Physiol Endocrinol Metab* 284:E177–E183