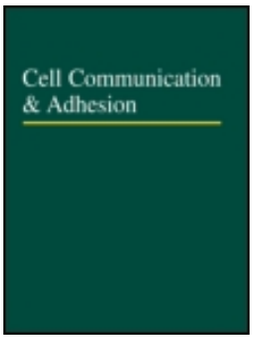

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General and Conditional Replacement of Connexin43-Coding DNA by a lacZ Reporter Gene for Cell-Autonomous Analysis of Expression

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Using the Cre/loxP system, we have circumvented early postnatal lethality and possible pleiotropic effects of general Cx43 gene deletion, in order to determine the expression and function of connexin43 (Cx43) in defined cell types. General or cell type-specific, Cre-mediated deletion of the floxed (i.e. flanked by loxP sites) Cx43-coding region led to activation of the inserted lacZ reporter gene in cells with transcriptional activity of the Cx43 gene. As deduced from lacZ expression in mice with general deletion, transcriptional activity of the Cx43 gene was not only found in a broad range of cell types known to a express Cx43, but also in pancreatic duct cells and vascular cells of the gut and skeletal muscle. Cre-mediated deletion restricted to defined cell types led to lacZ activation highlighting corresponding subsets of cells expressing Cx43, such as vascular endothelial cells, hepatic duct cells and putative neural crest cells, which were otherwise masked by strong Cx43 expression in neighbouring cells. In Cx43 expressing cell types, the floxed Cx43 allele was useful as a Cre-excision reporter for the characterization of Cre transgenes.

Keywords Cell autonomous, conditional replacement, Cre, Cx43 expression, loxP, reporter gene

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INTRODUCTION

Gap junctional intercellular channels allow for the intercellular diffusion of molecules up to 1 kDa and are formed by docking of two hemichannels contributed by neighbouring cells (Kumar and Gilula 1996). Hemichannels are comprised of six connexin subunits expressed by a gene family of at least 19 genes. Channels can be formed by similar or different types of hemichannels. Moreover, these can be comprised of either a uniform hexamer or of a mixture of connexins with different properties (Kumar and Gilula 1996), leading to a large variety of gap junctional intercellular channels. To determine the cell-type specificity of connexin gene expression, targeted replacements of connexin genes by a lacZ reporter gene have been generated in order to monitor transcriptional activity at the cellular level. Here we report on Cx43 expression using mice with a targeted replacement of the Cx43 coding region by a lacZ reporter gene ($Cx43^{del/+}$ mice; Theis et al. 2001). These mice were obtained by Cre-mediated, general replacement of a floxed Cx43 coding region ($Cx43^{fl}$ allele) by lacZ (Theis et al. 2001). The $Cx43^{fl}$ allele used in this study also allowed to detect the loss of Cx43 expression by gain of lacZ expression on endothelial cell specific, TIE2-Cre-mediated conditional replacement (Theis et al. 2001). As yet undiscovered sites of Cx43 expression were identified by lacZ expression after cell-type restricted, Cre-mediated recombination. We further demonstrate the usefulness of the $Cx43^{fl}$ allele as a Cre-excision reporter transgene for the characterization of Cre transgenes.

MATERIALS AND METHODS

Genotyping of TIE2-cre mice, $Cx43^{fl}$ mice, $Cx43^{+/-}$ mice and $Cx43^{del}$ mice (Theis et al. 2001), Flox lacZ indicator (FLZI) mice (Akagi et al. 1997), human glial fibrillary acidic protein promoter (hGFAP-) Cre mice (Zhuo et al. 2001) and rat insulin II promoter (INSPr-) Cre mice (Herrera 2000) was performed as previously described. X-Gal staining was performed as reported (Theis et al. 2001).

RESULTS AND DISCUSSION

The concept of lacZ activation following Cx43 deletion is shown in Figure 1A. Expression of the lacZ reporter gene inserted in the floxed allele occurred only in cells that had or still expressed Cre and showed transcriptional activity of the Cx43 gene (Figure 1B). In $Cx43^{del/+}$ mice with general deletion of Cx43, the pattern of lacZ expression, as visualized by X-gal staining, matched that expected for Cx43 expression from previous reports (Figure 2E, Campos de Carvalho et al. 1993; Theis et al. 2001),

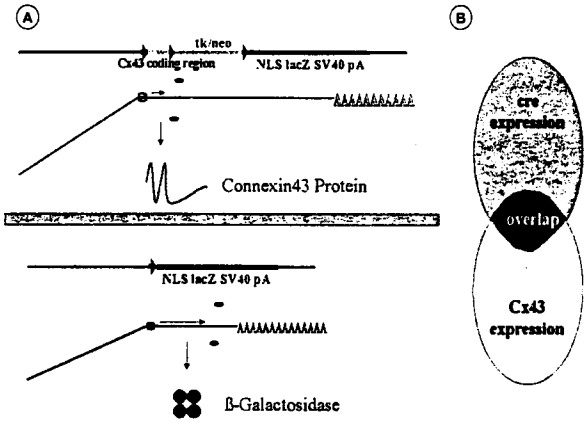

FIG. 1. lacZ activation on Cx43 deletion and transgenic set theory. A: Switch from Cx43 protein expression to lacZ expression. Prior to Cre-mediated recombination (upper part), a $Cx43^{fl}$ mRNA containing open reading frames (orf) for Cx43 and β-Galactosidase (β-Gal) is generated. Translation terminates at the Cx43 stop codon. Cre-mediated deletion of the Cx43 coding region and the selection marker cassette (tk/neo), encompassed by loxP sites (triangles), generates the $Cx43^{del}$ allele (lower part), carrying a lacZ gene in place of the Cx43 coding region. A $Cx43^{del}$ mRNA is generated, containing the orf for β-Gal, that is now translated. As a consequence, β-Gal, instead of Cx43, is expressed in cells with Cx43 gene activity. NLS: Nuclear localization sequence. pA: polyadenylation signal. AAA...: polyadenylated mRNA tail. Ellipses: ribosomes. B: Transgenic set theory. In mice with cell type-restricted deletion of the $Cx43^{fl}$ allele, lacZ expression only occurs in the overlap or common set (black area) of Cre expression (dark grey area) and Cx43 expression (light grey area). The set of Cx43 expressing cells can be visualized by general deletion of the $Cx43^{fl}$ allele and subsequent X-gal staining. The set of cells that express Cre or are clonal descendants of these cells, can be visualized by crossings with Cre-excision reporter mice, which ubiquitously express lacZ on Cre-mediated removal of a floxed STOP sequence.

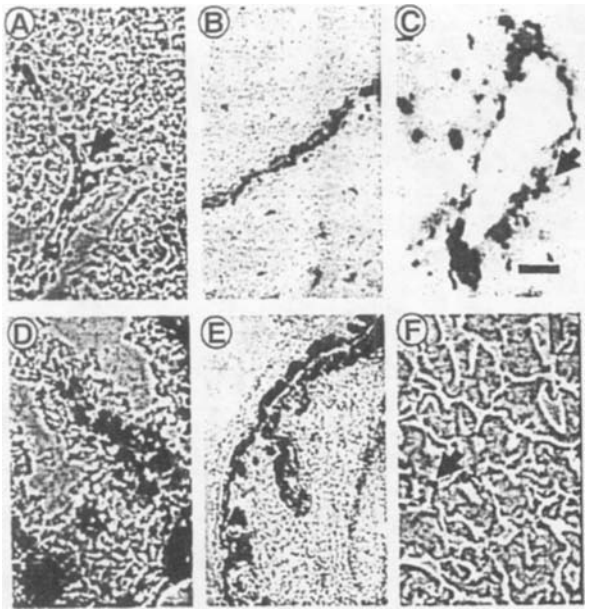

FIG. 2. Cx43 expression monitored by lacZ expression in cryosections of $Cx43^{del/+}$ mice. A–C: In the exocrine pancreas, lacZ expression was observed in cells of branched ducts (arrow in A), with either a single-layered (B) or a multilayered epithelium (arrow in C). D: Cx43 expression in the gut was observed in the highly vascularized lamina propria and the lamina muscularis mucosae. E: lacZ expression in the corpus cavernosum was observed in bundles of smooth muscle cells. F: lacZ expression in capillary endothelial cells (arrow) of skeletal muscle. Bar: 35 μm for A–D and F, and 140 μm for E.

with some exceptions, for example in the endocrine pancreas (Meda et al. 1993; Theis et al. 2001). Expression of Cx43 was also observed for the first time in epithelial duct cells of exocrine pancreas (Figure 2A–C) and in capillary endothelial cells of gut and skeletal muscle (Figure 2D, F). To further study this endothelial expression, we used a TIE2-Cre mediated activation of the lacZ reporter gene in the endothelial subset of Cx43 expression (Theis et al. 2001). Whole mount X-gal staining of the dorsal aorta of $Cx43^{fl/+}$, TIE2-Cre mice revealed Cx43 expression in the vasa vasorum that was masked in $Cx43^{del/+}$ mice by a much stronger expression of Cx43 in smooth muscle cells of the dorsal aorta (not shown). Thus, using a highly sensitive assay for transcriptional activity (lacZ) and a cell-type restricted detection of Cx43 expression (conditional replacement), we identified sites of Cx43 expression that had previously escaped immunodetection, due to low levels of Cx43 protein, or because these low levels were masked by the higher levels of Cx43 protein expressed by other types of nearby cells. Surprisingly, in mice with an insulin promoter (INSPr)-Cre mediated, beta-cell specific deletion of the $Cx43^{fl}$ allele, we did not observe lacZ expression in pancreatic islets (not shown). This was in contrast to the previously reported Cx43 expression in endocrine pancreas (Meda et al. 1993).

The exceptional strength of our experimental approach became apparent when we observed the INSPr-Cre mediated Cx43 deletion in a putative subpopulation of neural crest cells (Figure 3H). The rat insulin promoter fragment used in the INSPr-Cre transgene has previously been shown to be active in the neural tube during development (Alpert et al. 1988). Cre activity can be monitored using loxP bearing reporter mice such as the R26R (Soriano 1999) and FLZI strains (Akagi et al. 1997). Using INSPr-Cre, R26R mice, we were able to accurately analyze the expression of the INS promoter during development and in the adult (C. M. and P. H., unpublished results). We detected INSPr-Cre expression in various regions of the central nervous system (Figure 3G) as expected (Alpert et al. 1988). Interestingly, no lacZ staining was seen in the brain of INSPr-Cre × $Cx43^{fl/+}$ mice, indicating that Cx43 is not expressed in such regions (not shown). However, Cx43 is indeed expressed in neural crest-like cells of 13.5 dpc embryos (Figure 3H).

Without lacZ activation, the Cx43 expression in a subpopulation of putative neural crest cells would have remained undetected, i.e. masked by the abundant expression of Cx43 in other cell types (Theis et al. 2001). For this cell type, the $Cx43^{fl}$ allele was an alternative to Cre-excision reporter transgenes, since INSPr-Cre mediated recombination in neural crest cells was not monitored by the FLZI transgene (not shown). Also, lacZ expression in liver vanished shortly after recombination, when the FLZI transgene was used (Akagi et al. 1997). The R26R mouse showed hGFAP-Cre expression in epithelial duct cells of the liver besides strong expression in astrocytes (Zhuo et al. 2001). The lacZ inserted in the

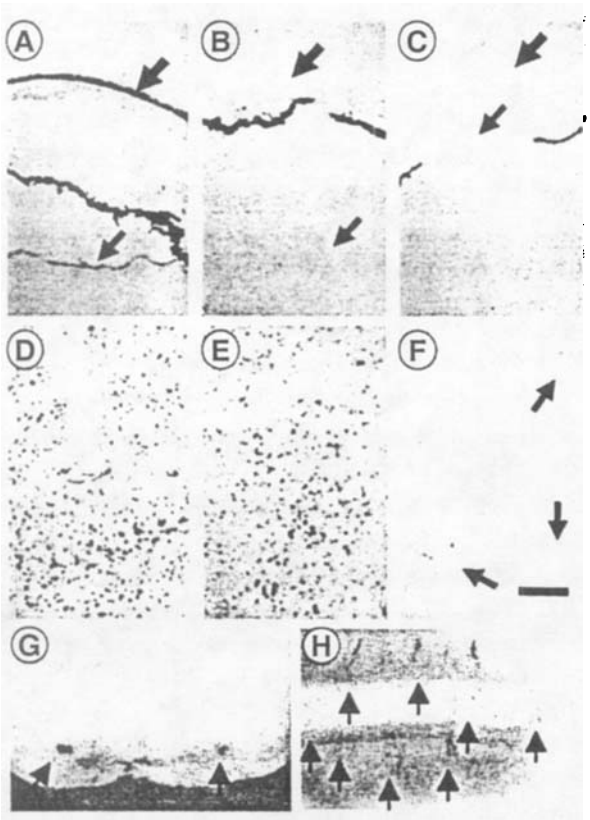

FIG. 3. Detection of Cx43 expression in small groups of cells and use of the $Cx43^{fl}$ allele as Cre-excision reporter. A, D: $Cx43^{del/+}$. B, E: $Cx43^{fl/+}$, TIE2-Cre. C, F: $Cx43^{fl/+}$, hGFAP-Cre. A–C: lacZ expression in cornea (large arrows) and lens epithelium (small arrows) occurs solely on general deletion (A), indicating lack of recombination mediated by the cell-type specific transgenes (B, C). D–F: Prominent lacZ expression was observed in nonparenchymal cells of liver on general deletion (D), and shown, using the TIE2-Cre transgene, to reflect mostly endothelial expression (E). A minority of Cx43 expressing cells (arrows), probably a subset of periportally located ductal cells was identified using hGFAP-Cre (F). G: Example of INS promoter activity in the central nervous system as revealed by X-gal staining. A strong lacZ expression is observed in the amygdaloid nucleus (arrows) of adult INSPr-Cre, R26R mice. H: Dorsal view of a $Cx43^{fl/+}$, INSPr-Cre embryo at 13.5 days post coitum. Arrows demarcate rows of lacZ-positive, putative neural crest cells expressing Cx43 and extending from the dorsal midline. Bar: 140 μm for A–F.

floxed allele also reported hGFAP-Cre expression in the liver. In this case, lacZ expression showed loss of Cx43 expression in a subpopulation of Cx43 expressing liver cells (Figure 3F) besides endothelial cells (Figure 3E) and other nonparenchymal liver cells (Figure 3D). On the other hand, the $Cx43^{fl}$ allele was useful to map the activity of Cre transgenes in Cx43 expressing cells. For example, strong lacZ expression was observed in lens epithelial cells of mice with general Cx43 deletion (Figure 3A), but not in mice with hGFAP-Cre mediated deletion (Figure 3C). This was unexpected, since GFAP had been detected in lens epithelial cells by Hatfield et al. (1985). We conclude that gene regulating elements which activate GFAP expression specifically in lens epithelial cells were not included in the promotor region used to drive Cre-expression in hGFAP-Cre mice. The same strategy for exclusion of Cre expression in lens and cornea worked for the TIE2-Cre gene (Figure 3B).

We conclude that the lacZ reporter gene is well-suited to monitor the activity of connexin genes. It provides the advantage of cell-autonomous analysis and of a higher sensitivity compared to immunodetection approaches. The lacZ reporter gene embedded in the $Cx43^{fl}$ allele has the additional advantage of monitoring expression in subsets of cells, thereby avoiding masking of weak signals, which is often encountered with *in situ* hybridization and immunodetection. The floxed Cx43 allele might also serve to characterize Cre transgenes where the Cre-excision reporter transgenes used are not suited for specific cell types.

REFERENCES

Akagi, K., et al. 1997. Cre-mediated somatic site-specific recombination in mice. *Nucleic Acids Research* 25:1766–1773.

Alpert, S., et al. 1988. Hybrid insulin genes reveal a developmental lineage for pancreatic endocrine cells and imply a relationship with neurons. *Cell* 53:295–308.

Campos de Carvalho, A. C., et al 1993. Gap junctions formed of connexin43 are found between smooth muscle cells of human corpus cavernosum. *J. Urology* 149:1568–1575.

Hatfield, J. S., et al. 1985. The lens epithelium contains glial fibrillary acidic protein (GFAP). *J. Neuroimmunology* 8:347–357.

Herrera, P. L. 2000. Adult insulin- and glucagon-producing cells differentiate from two independent cell lineages. *Development* 127:2317–2322.

Kumar, N. M., and Gilula, N. B. 1996. The gap junction communication channel. *Cell* 84:381–388.

Meda, P., et al. 1993. Differential expression of gap junction connexins in endocrine and exocrine glands. *Endocrinology* 133:2371–2378.

Soriano, P. 1999. Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nature Genetics* 21:70–71.

Theis, M., et al. 2001. Endothelium-specific replacement of the connexin43 coding region by a lacZ reporter gene. *Genesis* 29:1–13.

Zhuo, L., et al. 2001. hGFAP-cre transgenic mice for manipulation of glial and neuronal function in vivo. *Genesis* 31:85–94.