Establishment of a Tet-On Gene Expression System in Glucose-Responsive and -Unresponsive MIN6 Cells

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The tetracycline (or doxycycline)-inducible gene expression (Tet-On) system developed by Gossen and Bujard (1) is useful for controlling the expression of targeted genes and for determining the roles of the gene products in cellular functions. The Tet-On system has recently been applied to the rat insulinoma cell line INS-1 to study the roles of wild-type and mutant genes in insulin secretion (2,3). We have recently established two cell lines, MIN6-m9, and MIN6-m14, derived from the mouse insulinoma-derived cell line MIN6. MIN6-m9 and MIN6-m14 cells are glucose-responsive and glucose-unresponsive, respectively. To study the roles of newly identified genes in pancreatic β-cell functions, we have established a Tet-On system in these MIN6 sublines.

RESEARCH DESIGN AND METHODS
To establish stable cell lines that express the reverse tetracycline/doxycycline-responsive transcriptional activator (rtTA) and are capable of accessing transcription, MIN6-m9 cells and MIN6-m14 cells were transfected by electroporation with 40 µg pTet-On encoding rtTA. After selection with 400 µg/ml G418 for 28 days, resistant clones were selected by polymerase chain reaction (PCR) of genomic DNA and were tested for expression of rtTA and luciferase activity. Insulin-secretory responses to glucose and glibenclamide in MIN6-m9 Tet-On and MIN6-m14 Tet-On cells were determined in batch incubation experiments.

RESULTS
Of the five G418-resistant MIN6-m9 Tet-On cell lines selected by the PCR method, one subline, m9Tet-On 35, showed the highest increment (126-fold) in luciferase activity by transient transfection of pTRE-Luc, a reporter plasmid with the luciferase gene (maximal induction with 2 µg doxycycline). Of the 24 G418-resistant MIN6-m14 Tet-On cell lines selected, one subline, m14Tet-On 33, showed the highest increment (133-fold) in luciferase activity. Glucose-stimulated insulin secretion in m9Tet-On 35 occurred in a dose-dependent manner. Glibenclamide (100 nmol/l) also stimulated insulin secretion in m9Tet-On 35 cells. By contrast, there was almost no insulin response to either glucose or glibenclamide in m14Tet-On 33 cells. Thus, the features of m9Tet-On 35 cells and m14Tet-On 33 cells are similar to those of their parental glucose-responsive and -unresponsive MIN6 cells, respectively.

DISCUSSION
In the study of the role of ATP-dependent K+ (KATP) channels in pancreatic β-cell function at the cell level, m9Tet-On cells were secondarily transfected with the expression plasmid encoding a mouse Kir6.2 dominant-negative mutant (mKir6.2G132S). m9Tet-On cells expressing mKir6.2G132S are currently under selection with hygromycin. We will investigate the roles of the mutant KATP channel in cellular functions, including cell proliferation and death.

REFERENCES