Biomedical Research **32** (2) 167-174, 2011

Pancreatic β-cells are generated by neogenesis from non-β-cells after birth

Korefumi NAKAMURA[1], Kohtaro MINAMI[1], Kanako TAMURA[1], Keisuke IEMOTO[1], Takashi MIKI[1,*], and Susumu SEINO[1, 2, 3]

[1] Division of Cellular and Molecular Medicine, Department of Physiology and Cell Biology, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan; [2] Division of Diabetes and Endocrinology, Department of Internal Medicine, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan; and [3] Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Corp., Kawaguchi, Saitama 332-0012, Japan

(Received 14 January 2011; and accepted 31 January 2011)

ABSTRACT

The mass of pancreatic β-cells is maintained throughout lifetime to control blood glucose levels. Although the major mechanism of the maintenance of β-cell mass after birth is thought to be self-replication of pre-existing β-cells, it is possible that pancreatic β-cells are also generated from non-β-cells. Here, we address this issue by using the inducible Cre/loxP system to trace β-cells. We generated Ins2-CreERT2/R26R-YFP double knock-in mice, in which pancreatic β-cells can be labeled specifically and permanently upon injection of the synthetic estrogen analog tamoxifien, and then traced the β-cells by pulse and chase experiment in several different conditions. When β-cells were labeled in adults under physiological and untreated conditions, the frequency of the labeling (labeling index) was not altered significantly throughout the 12-month experimental period. In addition, the labeling index was not changed after ablation of β-cells by streptozotocin treatment. However, when tamoxifen was injected to pregnant mothers just before they gave birth, the labeling index in the neonates was decreased significantly around weaning, suggesting that β-cells are generated from non-β-cells. These results indicate that various mechanisms are involved in the maintenance of β-cells after birth, and that the present system using knock-in mice is useful for investigation of β-cell fate.

Pancreatic β-cells produce and secrete insulin, which is the only hormone that lowers blood glucose levels. The mass of the β-cells is not static but rather dynamic throughout lifetime (9, 19). The number of pancreatic β-cells increases in response to systemic insulin demand in obesity (18) and pregnancy (29), as well as in the neonatal period (17). It has been thought that β-cells are constantly replenished even under normal conditions (turnover) (9, 19). In general, adult tissues and organs are thought to be maintained by the stem cell system. The primary role of adult (tissue-specific) stem cells is to maintain and repair the tissue in which they reside. Although such stem cells have been found in several tissues including neurons (32), small intestine (5), and blood (15), both the existence and the nature of stem or progenitor cells in adult pancreas are yet to be established.

By using genetic cell lineage tracing, Dor *et al.* demonstrated that adult pancreatic β-cells in mice

Address correspondence to: Susumu Seino, M.D., D.M.Sci.
Division of Diabetes and Endocrinology, Department of Internal Medicine
Division of Cellular and Molecular Medicine, Department of Physiology and Cell Biology, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan
Tel: +81-78-382-5860, Fax: +81-78-382-6762
E-mail: seino@med.kobe-u.ac.jp

*Present address: Department of Medical Physiology, Graduate School of Medicine, Chiba University, Chiba 260-8670, Japan

are maintained predominantly by self-replication of pre-existing β-cells (6). Furthermore, another study using a DNA analog-based lineage-tracing technique showed that unlike gastrointestinal and skin epithelia, specialized progenitors do not contribute to adult β-cell mass, even during acute β-cell regeneration (33). Instead, adult β-cells exhibit equal proliferation potential, and expand from a vast and uniform pool of mature β-cells (3). These findings support the notion that the mass of pancreatic β-cells is maintained primarily by self-replication of pre-existing β-cells. On the other hand, there are many studies suggesting generation of pancreatic β-cells from non-β-cells (Ref. 21 for review). Importantly, Xu *et al.* made use of a unique model of tissue damage and found that cells expressing Ngn3, which represent progenitors for endocrine cells during embryogenesis, reappeared in adult mice following injury and gave rise to new β-cells (36). This study provides evidence that facultative β-cell progenitors exist, although definitive identification of the cell type having progenitor property remains elusive. Extensive analysis of the formation of new pancreatic β-cells is required to elucidate the mechanisms of the maintenance of β-cell mass.

In the present study, we newly generated a knock-in mouse model, in which the endogenous mouse insulin 2 gene is replaced with a gene of Cre-recombinase and modified estrogen receptor fusion protein (CreERT2). By crossbreeding these mice with a reporter strain expressing yellow fluorescent protein (YFP) by Cre-mediated loxP recombination (R26R-YFP) (30), we established a system for tracing pancreatic β-cells upon tamoxifen injection. We analyzed the fate of pancreatic β-cells in the Ins2-CreERT2/R26R-YFP double knock-in mice in three different settings. Our data indicate that new β-cells are generated from non-β-cells after birth.

MATERIALS AND METHODS

Generation of Ins2-CreERT2/R26R-YFP mice. All animal experiments were approved by the Animal Research Committee of Kobe University Graduate School of Medicine. Ins2-CreERT2 knock-in mice were generated by replacing the mouse insulin 2 gene with a CreERT2 cassette, which was inserted into the ATG site of the gene located in exon 2, by homologous recombination (Fig. 1A). Genotyping of Ins2-CreERT2 knock-in mice was performed by PCR with primers for mouse insulin 2 and CreERT2 (forward for both genotypes: 5'-CCCTAAGTGATC CGCTACAA-3', reverse for wild type: 5'-CTTGTG GGTCCTCCACTTCA-3', and reverse for CreERT2: 5'-CAGCATTGCTGTCACTTGGT-3'). The amplicons were 362 bp for the wild type allele and 512 bp for the targeted allele (Fig. 1A and B). Ins2-CreERT2 knock-in mice were crossed with R26R-YFP mice (provided by F. Costantini, Columbia University, New York) (30) to generate Ins2-CreERT2/R26R-YFP mice for tracing pancreatic β-cells.

Labeling β-cells by tamoxifen injection. For lineage tracing in adult pancreas, Ins2-CreERT2/R26R-YFP mice of 6 weeks of age were injected intraperitoneally with tamoxifen (Sigma, St. Louis, MO) five times (6 mg/head for the first time and 4 mg/head for the remaining) within two weeks. For tracing in neonates, pregnant mothers of Ins2-CreERT2/R26R-YFP mice were injected intraperitoneally with a single dose of 6 mg/30 g body weight of 4-hydroxy-tamoxifen (Sigma) on the day before they gave birth.

Streptozotocin treatment. For tracing β-cells after a severe injury, Ins2-CreERT2/R26R-YFP mice were injected intraperitoneally with streptozotocin (STZ) (Sigma) at 100 mg/kg body weight 10 days after tamoxifen treatment as described above. Mice with blood glucose concentration above 300 mg/dL were used for the study.

Immunohistochemistry. Pancreata removed from the mice were fixed in 4% paraformaldehyde. Frozen sections were stained with antibodies against insulin (Zymed, San Francisco, CA) (1 : 100), Cre (Novagen, Madison, WI) (1 : 10,000), GFP (Molecular Probes, Eugene, OR) (1 : 200), and Ki67 (Dako Japan, Tokyo, Japan) (1 : 500). Secondary antibodies conjugated with Alexa Fluor 488- or 546 (1 : 400) (Molecular Probes) were used for detection. Nuclei were visualized by DAPI (Dojindo, Kumamoto, Japan). Because YFP is one of the emission variants of GFP in which only four amino acids are substituted from GFP, antibodies against GFP can recognize this protein (20). The stained sections were observed with BZ9000 microscope (Keyence, Osaka, Japan). The frequency of the labeling (labeling index) of the pancreatic β-cells was calculated by dividing the number of YFP-positive cells by the number of insulin-positive cells. For measurement of β-cell mass, we first measured percent area of β-cells in the pancreas from five distinct sections of each mouse pancreas. The β-cell mass was then estimated by multiplying the β-cell area by the pancreas weight of corresponding animals. All data are means of the

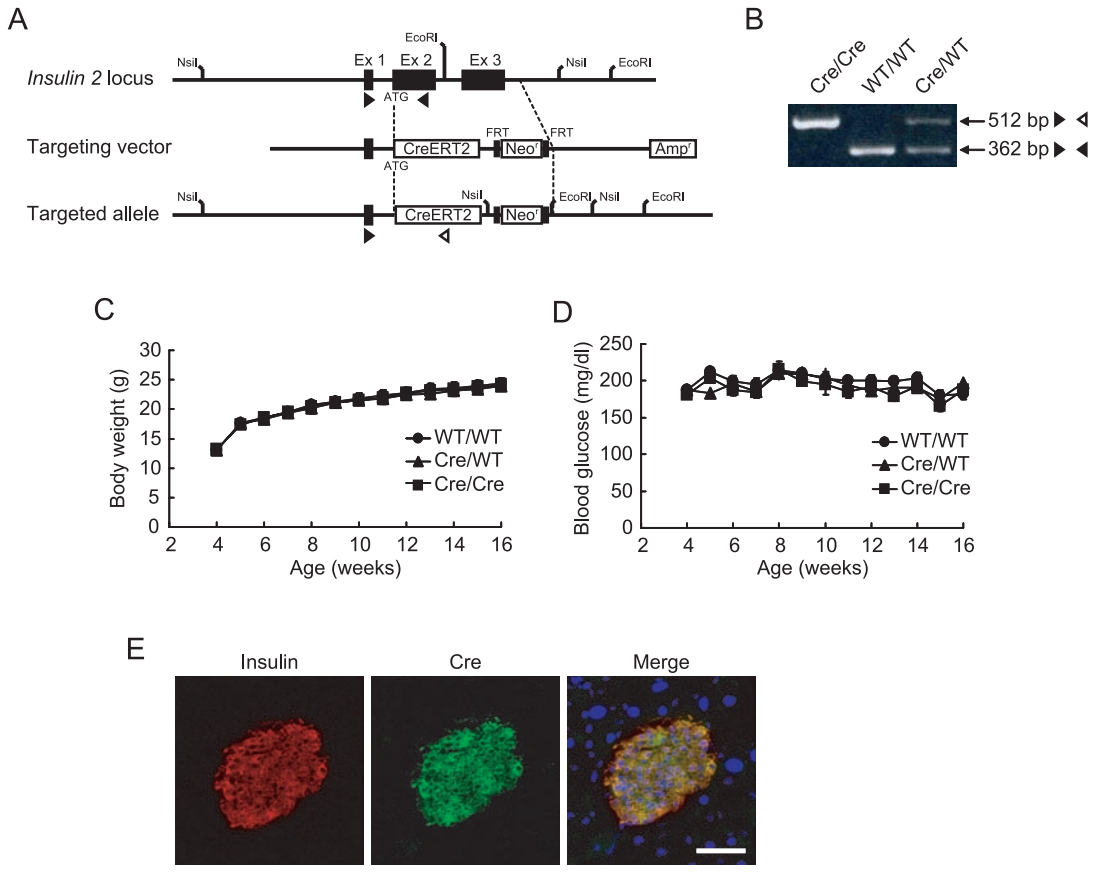

Fig. 1 Generation of Ins2-CreERT2 knock-in mice. (**A**) Schematic representation of mouse insulin 2 gene, targeting vector, and targeted allele. A CreERT2 cassette was inserted into the ATG site of the gene located in exon 2 of mouse insulin 2 gene. Triangles indicate primers used for genotyping. (**B**) Genomic PCR analysis for Ins2-CreERT2 knock-in mice. Triangles in (B) represent primers used for PCR, which correspond to those seen in (A). (**C** and **D**) Body weight (C) and casual blood glucose levels (D) of each genotype. There were no significant differences among these groups. (**E**) Immunohistochemistry of pancreas of Ins2-CreERT2 knock-in mice for insulin and Cre-recombinase. All of the Cre-positive cells were also positive for insulin. Scale bar, 50 μm.

results from five mice.

Statistical analysis. Values are expressed as means ± S.E. The significance of difference between test groups was evaluated by use of multiple analysis of Tukey-Kramer's test. $P < 0.05$ was considered significant.

RESULTS

Generation of Ins2-CreERT2/R26R-YFP double knock-in mice

We first generated Ins2-CreERT2 knock-in mice, in which a fusion protein containing Cre-recombinase and modified estrogen receptor is replaced with mouse insulin 2 gene (Fig. 1A). Genotypes of the mice were determined by genomic PCR (Fig. 1B). Unlike conventional transgenic mice expressing Cre-recombinase driven by insulin promoter, expression of Cre-recombinase in the Ins2-CreERT2 knock-in mice perfectly reproduces insulin expression and no position effect is caused in the mice. We confirmed that body weight and casual blood glucose levels of the homozygous mice were identical to those of the heterozygous and wild-type mice (Fig. 1C and D). Expression of Cre-recombinase was detected only in insulin-expressing β-cells (Fig. 1E). We then crossbred the Ins2-CreERT2 knock-in mice with R26R-YFP mice (30) to generate Ins2-CreERT2/R26R-YFP double knock-in mice (Fig. 2A). Upon injection of tamoxifen as described in MATERIALS AND METHODS, expression of YFP was detected exclusively in insulin-expressing β-cells (Fig. 2B). These results demonstrate that Ins2-CreERT2/R26R-YFP double knock-in mice work well for tracing pancreatic β-cell fate.

Tracing pancreatic β-cells with aging under normal condition

Using Ins2-CreERT2/R26R-YFP double knock-in mice, we chased pancreatic β-cells in several settings. Firstly, we investigated the β-cells in adult under normal condition (Fig. 3A). We injected tamoxifen five times to the mice for two weeks starting at 6 weeks of age. Three days after the last injection (pulse), pancreata of these mice were dissected and subjected to immunostaining for YFP and insulin to determine labeling index. We found that 25.8 ± 3.75% of insulin-positive cells were labeled with YFP (Fig. 3B and C). The labeling index was not changed significantly throughout the 12-month ex-

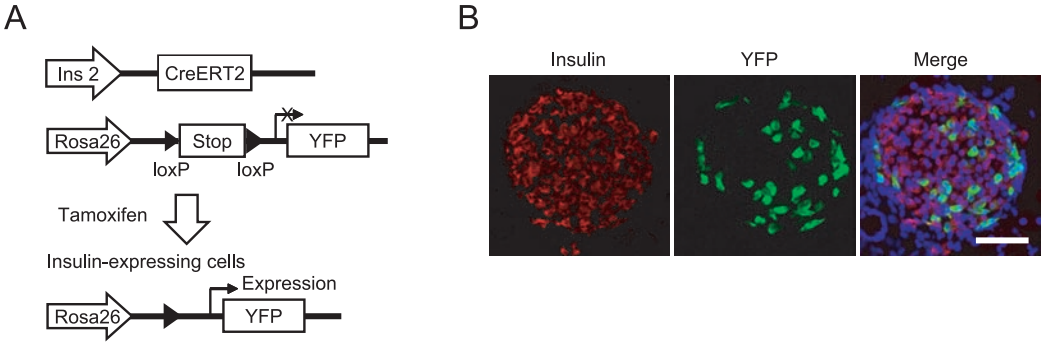

Fig. 2 Generation of Ins2-CreERT2/R26R-YFP double knock-in mice. (**A**) Experimental strategy of inducible Cre-mediated tracing of pancreatic *β*-cells. Ins2-CreERT2/R26R-YFP double knock-in mice were generated by crossbreeding of Ins2-CreERT2 mice and R26R-YFP mice. Upon infection of tamoxifen, insulin-expressing cells (*β*-cells) begin to express YFP and are permanently labeled with the fluorescent protein. (**B**) Double immunostaining of pancreas of Ins2-CreERT2/R26R-YFP double knock-in mice for insulin and YFP after injection of tamoxifen. Expression of YFP was highly restricted in insulin-positive cells. Scale bar, 50 μm.

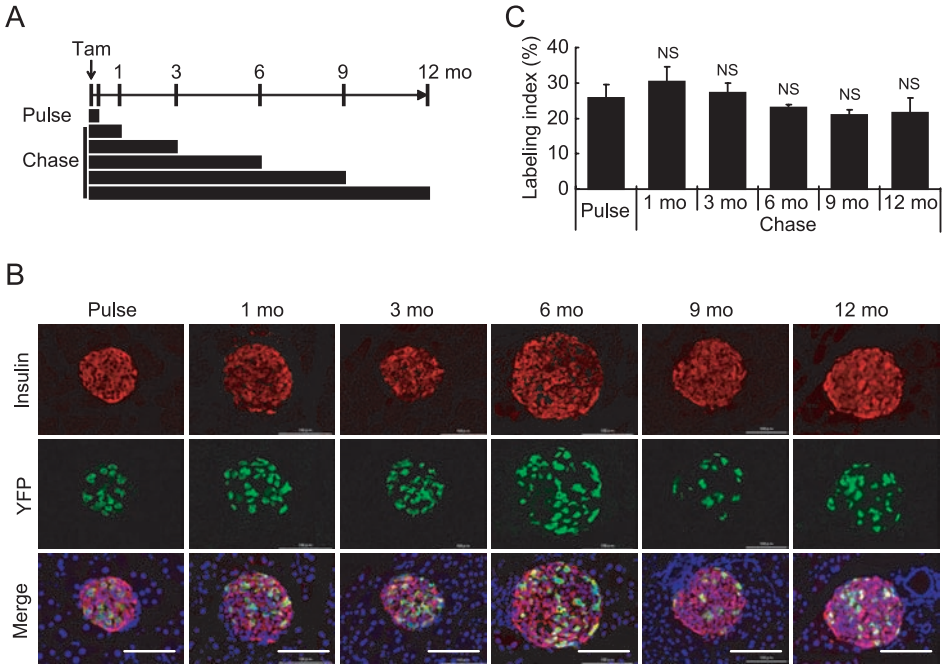

Fig. 3 Tracing *β*-cells with aging. (**A**) Outline of experimental design. Tamoxifen was injected five times to Ins2-CreERT2/R26R-YFP double knock-in mice from 6 to 8 weeks of age to label *β*-cells. Three days after the last injection represents the "Pulse" period. The *β*-cells were chased up to 12 months after the treatment. (**B**) Double immunostaining for insulin and YFP in pancreas of the mice after tamoxifen injection. Scale bars, 100 μm. (**C**) Quantification of YFP-labeled *β*-cells. Change in the labeling index (percentage of the number of YFP-positive cells among insulin-positive cells) is shown. When compared to the pulse period, the index of each time point did not differ significantly. NS, difference not significant vs. pulse period.

perimental period (Fig. 3B and C). This result is consistent with the previous finding by Dor *et al*. (6). Our data support the notion that the major mechanism of the maintenance of adult pancreatic β-cells may be self-replication rather than neogenesis from stem/progenitors under normal condition, as reported previously (6, 10).

Tracing pancreatic β-cells after β-cell ablation

Although β-cell mass is maintained by self-replication under normal condition, it has been suggested that neogenesis from stem or progenitor cells could contribute to generation of new β-cells after injury (22). The β-cell toxin streptozotocin (STZ) is known to cause rapid and severe β-cell damage. In some studies, it has been found that insulin-positive cells reappear after the loss of pancreatic β-cells by STZ treatment (8, 13). Therefore, we examined pancreatic β-cell fate after STZ treatment to investigate whether β-cells might be generated from non-β-cells (Fig. 4A). Pancreatic β-cells of Ins2-CreERT2/R26R-YFP double knock-in mice were labeled by tamoxifen injection 10 days before STZ treatment (100 mg/kg, i.p.). This dose of STZ caused a moderate loss of pancreatic β-cells (Fig. 4B). We traced the β-cells for up to 3 months after STZ treatment. The labeling index of these β-cells was unchanged throughout the experiment (Fig. 4C), indicating that neogenesis of β-cells from non-β-cells did not occur under the conditions used. While we failed to detect replenishment of β-cells after treatment of STZ, further examination using other models of β-cell regeneration are required to clarify whether neogenesis participates in regeneration of pancreatic β-cells.

Tracing pancreatic β-cells in neonate

The mass of pancreatic β-cells increases rapidly within a month after birth (7). It has been thought that the increase in β-cell mass depends largely on replication of pre-existing β-cells during this time (9, 17). If this is the case, the labeling index in Ins2-CreERT2/R26R-YFP double knock-in mice should be unchanged in this period. We injected 4-hydorxytamoxifen to pregnant mothers of the mice one day before they gave birth (Fig. 5A). The frequency of the labeling of pancreatic β-cells of new born mice

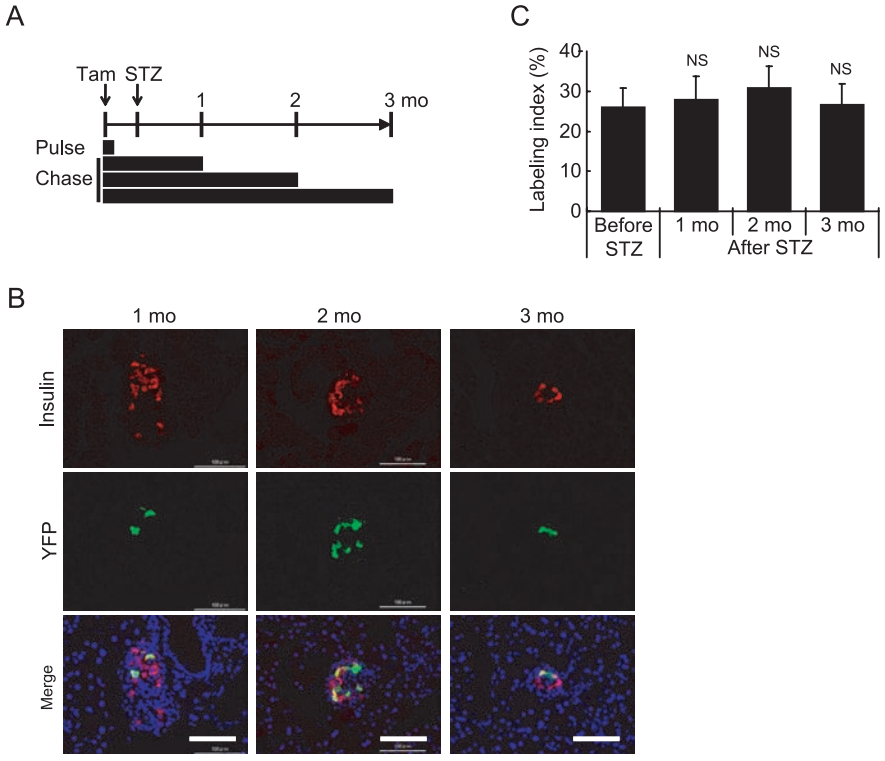

Fig. 4 Tracing *β*-cells under *β*-cell ablation. (**A**) Outline of experimental design. Tamoxifen was injected to Ins2-CreERT2/R26R-YFP double knock-in mice as described and the mice were treated with STZ. The *β*-cells were chased up to 3 months after STZ treatment. (**B**) Double immunostaining for insulin and YFP in pancreas of the mice after STZ treatment. Scale bars, 100 μm. (**C**) Quantification of YFP-labeled *β*-cells. Change in the labeling index is shown. When compared to the pulse period, the index of each time point did not differ significantly. NS, difference vs. pulse period was not significant.

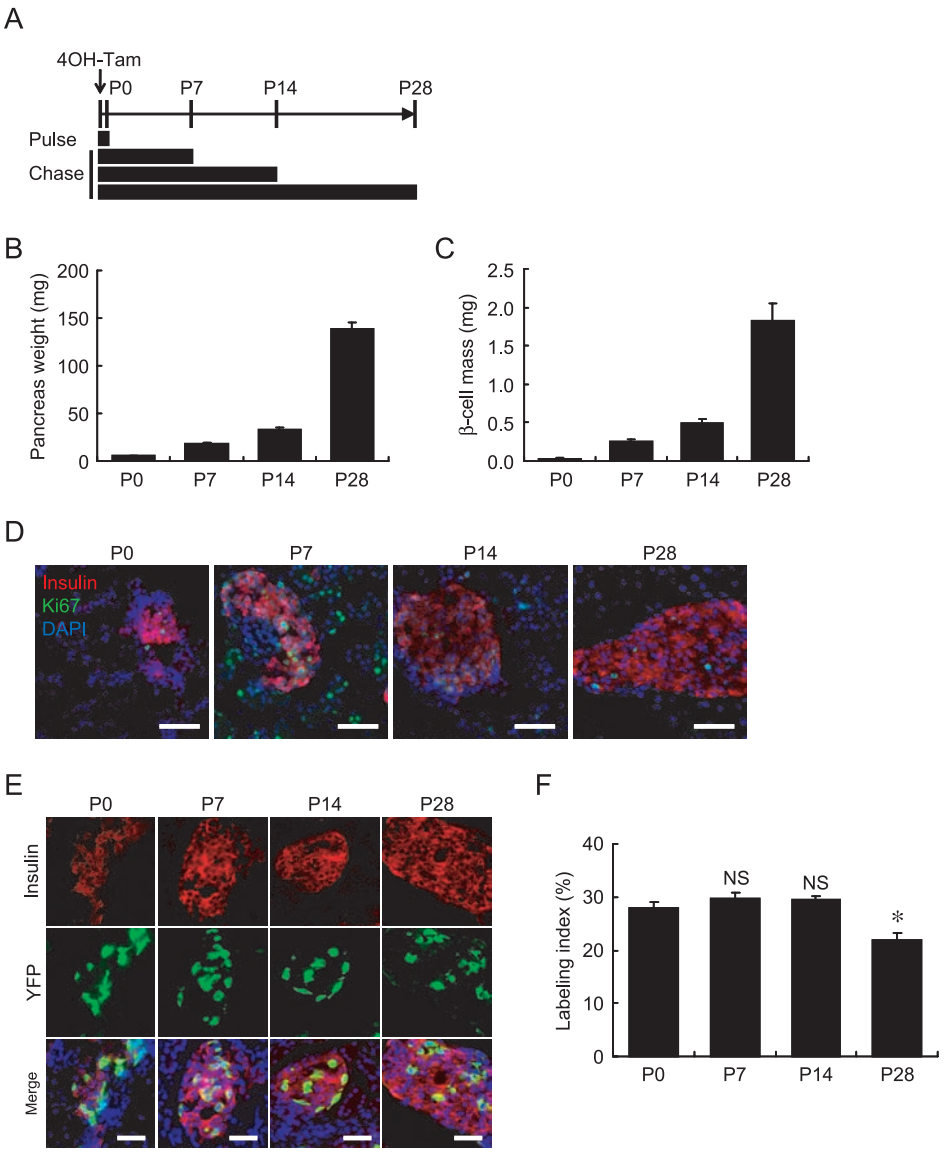

Fig. 5 Tracing β-cells during neonatal period. (**A**) Outline of experimental design. Tamoxifen was injected to pregnant mothers of Ins2-CreERT2/R26R-YFP double knock-in mice the day before they gave birth. The β-cells were chased for a month. (**B**–**D**) Changes in pancreatic weight (B) and β-cell mass (C) in pancreas of the neonates. (D) Double immunostaining for insulin and Ki67 in pancreas of the neonates. A considerable number of insulin/Ki67-double positive cells was detected. Scale bars, 50 µm. (**E**) Double immunostaining for insulin and YFP in pancreas of the neonates. Scale bars, 100 µm. (**F**) Quantification of YFP-labeled β-cells. NS, difference not significant. $^*P < 0.05$ vs. pulse period.

(P0) was 28.0 ± 0.95% of total β-cells. We confirmed that the pancreas weight of the mice was increased (Fig. 5B). The mass of β-cells was also increased, especially between postnatal day 14 (P14) and P28 (Fig. 5C). This is consistent with a previous study in rats showing that a significant increase in β-cell mass was seen after P20 (25). Proliferating cell marker Ki67-positive cells could readily be detected (Fig. 5D), suggesting that massive replication of the β-cells occurs during this period (Fig. 5E). The labeling index of pancreatic β-cells in the mice was unchanged from P0 to P14 (Fig. 5F). However, the index significantly decreased at P28 (Fig. 5F), suggesting the generation of new β-cells by other mechanisms than replication of pre-existing β-cells in this period.

DISCUSSION

Several studies have shown that adult mouse pan-

creas does not require progenitors for β-cells (6, 10, 33). In addition, the size of the pancreas depends on the number of embryonic progenitor cells, indicating that there is no contribution of postnatal progenitors to growth of the pancreas (31). Furthermore, a recent paper demonstrated that pancreatic duct cells, which have been thought to contain the progenitor pool, do not give rise to β-cells after birth (26). These studies strongly suggest that adult pancreatic β-cells are maintained by self-replication of pre-existing β-cells, and that there be no stem or progenitor cells of β-cells in the adult pancreas.

However, in the adult pancreas, the generation of β-cells from non-β-cells under certain conditions is also suggested (11, 12, 14, 28, 35, 36). For example, Hao *et al.* have shown that non-endocrine epithelial cells of the adult human pancreas differentiate into β-cells when transplanted under the renal capsule (14). *In vitro* generation of β-cells from non-β-cells has also been reported by many laboratories (1, 2, 20, 24, 27). These results clearly indicate that there are cells having potential to generate β-cells in the adult pancreas. Moreover, Xu *et al.* reported that progenitors for β-cells reside in adult pancreas and can be activated by injury to generate all islet cell types including β-cells *in vivo* (36). We also have shown that pancreatic β-cells of transgenic mice expressing a dominant-negative form of Kir6.2, a subunit of the β-cell-type ATP-sensitive K^+ channel, were spontaneously regenerated from *Dolichos biflorus* agglutinin-labeled cells that appeared within the pancreatic islets (23). Inada *et al.* reported that carbonic anhydrase II-positive cells within the pancreas act as progenitors that give rise to new islets (16). Very recent papers indicated that pancreatic β-cells can be generated from α-cells under conditions of severe ablation of β-cells (4, 34).

Considering these findings together, mechanisms of generation of new pancreatic β-cells other than self-replication of pre-existing β-cells may well operate in adult pancreas. Indeed, we have shown here that labeling index of pancreatic β-cells was decreased significantly from P14 to P28 (Fig. 5), the period during which the β-cell mass was markedly increased, indicating that new β-cells are generated by both self-replication of pre-existing β-cells and neogenesis from stem or progenitor cells. As this period corresponds to that of weaning, alterations in nutritional status may be related to the phenomenon. Further investigation of the neogensis of pancreatic β-cells occurring around weaning is required to elucidate the fate of β-cells in the postnatal period.

Acknowledgements

We thank Ritsuko Hoshikawa for technical assistance. This work was supported by a CREST grant from the Japan Science and Technology Agency and Grant-in-Aid for Scientific Research and grants for the Global Center of Excellence (COE) Programs "Global Center of Excellence for Education and Research on Signal Transduction Medicine in the Coming Generation", "Global Center for Education and Research in Integrative Membrane Biology", Kobe Translational Research Cluster, the Knowledge Cluster Initiative from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and a grant from Suzuken Memorial Foundation.

REFERENCES

1. Baeyens L, De Breuck S, Lardon J, Mfopou JK, Rooman I and Bouwens L (2005) *In vitro* generation of insulin-producing beta cells from adult exocrine pancreatic cells. *Diabetologia* **48**, 49–57.
2. Bonner-Weir S, Taneja M, Weir GC, Tatarkiewicz K, Song KH, Sharma A and O'Neil JJ (2000) *In vitro* cultivation of human islets from expanded ductal tissue. *Proc Natl Acad Sci U S A* **97**, 7999–8004.
3. Brennand K, Huangfu D and Melton D (2007) All beta cells contribute equally to islet growth and maintenance. *PLoS Biol* **5**, e163.
4. Chung CH, Hao E, Piran R, Keinan E and Levine F (2010) Pancreatic β-cell neogenesis by direct conversion from mature α-cells. *Stem Cells* **28**, 1630–1638.
5. Crosnier C, Stamataki D and Lewis J (2006) Organizing cell renewal in the intestine: stem cells, signals and combinatorial control. *Nat Rev Genet* **7**, 349–359.
6. Dor Y, Brown J, Martinez OI and Melton DA (2004) Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* **429**, 41–46.
7. Dore BA, Grogan WM, Madge GE and Webb SR (1981) Biphasic development of the postnatal mouse pancreas. *Biol Neonate* **40**, 209–217.
8. Fernandes A, King LC, Guz Y, Stein R, Wright CV and Teitelman G (1997) Differentiation of new insulin-producing cells is induced by injury in adult pancreatic islets. *Endocrinology* **138**, 1750–1762.
9. Finegood DT, Scaglia L and Bonner-Weir S (1995) Dynamics of beta-cell mass in the growing rat pancreas. Estimation with a simple mathematical model. *Diabetes* **44**, 249–256.
10. Georgia S and Bhushan A (2004) Beta cell replication is the primary mechanism for maintaining postnatal beta cell mass. *J Clin Invest* **114**, 963–968.
11. Gu D, Lee MS, Krahl T and Sarvetnick N (1994) Transitional cells in the regenerating pancreas. *Development* **120**, 1873–1881.
12. Gu D and Sarvetnick N (1993) Epithelial cell proliferation and islet neogenesis in IFN-g transgenic mice. *Development* **118**, 33–46.
13. Guz Y, Nasir I and Teitelman G (2001) Regeneration of pancreatic beta cells from intra-islet precursor cells in an experimental model of diabetes. *Endocrinology* **142**, 4956–4968.
14. Hao E, Tyrberg B, Itkin-Ansari P, Lakey JR, Geron I,

Monosov EZ, Barcova M, Mercola M and Levine F (2006) Beta-cell differentiation from nonendocrine epithelial cells of the adult human pancreas. *Nat Med* **12**, 310–316.
15. Huang X, Cho S and Spangrude GJ (2007) Hematopoietic stem cells: generation and self-renewal. *Cell Death Differ* **14**, 1851–1859.
16. Inada A, Nienaber C, Katsuta H, Fujitani Y, Levine J, Morita R, Sharma A and Bonner-Weir S (2008) Carbonic anhydrase II-positive pancreatic cells are progenitors for both endocrine and exocrine pancreas after birth. *Proc Natl Acad Sci U S A* **105**, 19915–19919.
17. Kaung HL (1994) Growth dynamics of pancreatic islet cell populations during fetal and neonatal development of the rat. *Dev Dyn* **200**, 163–175.
18. Klöppel G, Löhr M, Habich K, Oberholzer M and Heitz PU (1985) Islet pathology and the pathogenesis of type 1 and type 2 diabetes mellitus revisited. *Surv Synth Pathol Res* **4**, 110–125.
19. Matveyenko AV, Veldhuis JD and Butler PC (2008) Adaptations in pulsatile insulin secretion, hepatic insulin clearance, and beta-cell mass to age-related insulin resistance in rats. *Am J Physiol Endocrinol Metab* **295**, E832–E841.
20. Minami K, Okuno M, Miyawaki K, Okumachi A, Ishizaki K, Oyama K, Kawaguchi M, Ishizuka N, Iwanaga T and Seino S (2005) Lineage tracing and characterization of insulin-secreting cells generated from adult pancreatic acinar cells. *Proc Natl Acad Sci U S A* **102**, 15116–15121.
21. Minami K and Seino S (2008) Pancreatic acinar-to-beta cell transdifferentiation in vitro. *Front Biosci* **13**, 5824–5837.
22. O'Neill KE, Eberhard D and Tosh D (2008) Origin of beta-cells in regenerating pancreas. *Bioessays* **30**, 617–620.
23. Oyama K, Minami K, Ishizaki K, Fuse M, Miki T and Seino S (2006) Spontaneous recovery from hyperglycemia by regeneration of pancreatic beta-cells in Kir6.2G132S transgenic mice. *Diabetes* **55**, 1930–1938.
24. Ramiya VK, Maraist M, Arfors KE, Schatz DA, Peck AB and Cornelius JG (2000) Reversal of insulin-dependent diabetes using islets generated in vitro from pancreatic stem cells. *Nat Med* **6**, 278–282.
25. Scaglia L, Cahill CJ, Finegood DT and Bonner-Weir S (1997) Apoptosis participates in the remodeling of the endocrine pancreas in the neonatal rat. *Endocrinology* **138**, 1736–1741.
26. Solar M, Cardalda C, Houbracken I, Martín M, Maestro MA, De Medts N, Xu X, Grau V, Heimberg H, Bouwens L and Ferrer J (2009) Pancreatic exocrine duct cells give rise to insulin-producing beta cells during embryogenesis but not after birth. *Dev Cell* **17**, 849–860.
27. Song KH, Ko SH, Ahn YB, Yoo SJ, Chin HM, Kaneto H, Yoon KH, Cha BY, Lee KW and Son HY (2004) *In vitro* transdifferentiation of adult pancreatic acinar cells into insulin-expressing cells. *Biochem Biophys Res Commun* **316**, 1094–1100.
28. Song SY, Gannon M, Washington M K, Scoggins CR, Meszoely IM, Goldenring JR, Marino CR, Sandgren EP, Coffey RJ Jr., Wright CV and Leach SD (1999) Expansion of Pdx1-expressing pancreatic epithelium and islet neogenesis in transgenic mice overexpressing transforming growth factor alpha. *Gastroenterology* **117**, 1416–1426.
29. Sorenson RL and Brelje TC (1997) Adaptation of islets of Langerhans to pregnancy: beta-cell growth, enhanced insulin secretion and the role of lactogenic hormones. *Horm Metab Res* **29**, 301–307.
30. Srinivas S, Watanabe T, Lin CS, William CM, Tanabe Y, Jessell TM and Costantini F (2001) Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC Dev Biol* **1**, 4.
31. Stanger BZ, Tanaka AJ and Melton DA (2007) Organ size is limited by the number of embryonic progenitor cells in the pancreas but not the liver. *Nature* **445**, 886–891.
32. Temple S (2001) The development of neural stem cells. *Nature* **414**, 112–117.
33. Teta M, Rankin MM, Long SY, Stein GM and Kushner JA (2007) Growth and regeneration of adult beta cells does not involve specialized progenitors. *Dev Cell* **12**, 817–826.
34. Thorel F, Népote V, Avril I, Kohno K, Desgraz R, Chera S and Herrera PL (2010) Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. *Nature* **464**, 1149–1154.
35. Wang RN, Klöppel G and Bouwens L (1995) Duct- to islet-cell differentiation and islet growth in the pancreas of duct-ligated adult rats. *Diabetologia* **38**, 1405–1411.
36. Xu X, D'Hoker J, Stangé G, Bonné S, De Leu N, Xiao X, Van de Casteele M, Mellitzer G, Ling Z, Pipeleers D, Bouwens L, Scharfmann R, Gradwohl G and Heimberg H (2008) Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. *Cell* **132**, 197–207.