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Cyclins D2 and D1 Are Essential for Postnatal Pancreatic β-Cell Growth

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Regulation of adult β-cell mass in pancreatic islets is essential to preserve sufficient insulin secretion in order to appropriately regulate glucose homeostasis. In many tissues mitogens influence development by stimulating D-type cyclins (D1, D2, or D3) and activating cyclin-dependent kinases (CDK4 or CDK6), which results in progression through the G_1 phase of the cell cycle. Here we show that cyclins D2 and D1 are essential for normal postnatal islet growth. In adult murine islets basal cyclin D2 mRNA expression was easily detected, while cyclin D1 was expressed at lower levels and cyclin D3 was nearly undetectable. Prenatal islet development occurred normally in *cyclin D2*$^{-/-}$ or *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice. However, β-cell proliferation, adult mass, and glucose tolerance were decreased in adult *cyclin D2*$^{-/-}$ mice, causing glucose intolerance that progressed to diabetes by 12 months of age. Although *cyclin D1*$^{+/-}$ mice never developed diabetes, life-threatening diabetes developed in 3-month-old *cyclin D1*$^{-/+}$ *D2*$^{-/-}$ mice as β-cell mass decreased after birth. Thus, cyclins D2 and D1 were essential for β-cell expansion in adult mice. Strategies to tightly regulate D-type cyclin activity in β cells could prevent or cure diabetes.

Insufficient insulin secretion and inadequate β-cell growth are central components in the pathogenesis of type I and type II diabetes (1, 4, 20, 28, 31). Several mechanisms have been proposed to explain how new β cells are formed, including replication of preexisting β cells and neogenesis from putative precursors (2, 3, 7, 17). Many factors influence adult β-cell growth, function and survival, including signals generated through the Irs2 branch of the insulin/IGF signaling cascade (14, 29, 30). Mitogens stimulate progression through the G_1 phase of cell cycle, at least in part by stimulating *D-type cyclin* gene expression and/or increasing D-type cyclin protein accumulation or activity (22). D-type cyclins associate with cdk4 or cdk6, which stimulates the kinase to phosphorylate and inactivate the retinoblastoma tumor suppressor protein (pRB) and pRB-related proteins p107 and p130 (22). The activated cyclin D/cdk4 or -6 complex also promotes cell cycle progression by sequestering the cyclin E-cdk2 and cyclin A-cdk2 complex kip inhibitors ($p21^{CIP1}$, $p27^{KIP1}$, and $p57^{KIP2}$), which increases cdk2-associated activity in late G_1 and S phase (22). Moreover, $p27^{kip}$ is a principal cell cycle inhibitor in β cells, as it accumulates in the nucleus of β cells from obese mice, inhibiting compensatory β-cell expansion (27).

Three structurally similar D-type cyclins (D1, D2, and D3) are expressed in an overlapping and redundant fashion. However, germ line ablation of the *D-type cyclin* genes reveals tissue specificity for these cyclin isoforms. *cyclin D1*$^{-/-}$ mice are small, with impaired neuronal, retinal, and mammary gland development (8, 25). *cyclin D2*$^{-/-}$ mice display cerebellar abnormalities and impaired proliferation of B lymphocytes (24). Female *cyclin D2*$^{-/-}$ mice are infertile due to the inability of the ovarian granulosa cells to respond normally to follicle-stimulating hormone, whereas male *cyclin D2*$^{-/-}$ mice are fertile but have hypoplastic testes (24). *cyclin D3*$^{-/-}$ mice display abnormalities in hematological development (23). Despite these abnormalities, D-type cyclin-deficient mice (missing cyclin D1, D2, or D3) are fairly healthy and have normal life spans. Mice lacking cdk4, which is activated during association with a D-type cyclin, display somatic growth defects similar to those of *cyclin D1*$^{-/-}$ mice, infertility like *cyclin D2*$^{-/-}$ mice, and inadequate islet expansion that progresses to diabetes (21, 26). By contrast, constitutively active cdk4 introduced into the mouse genome—by substitution of Arg-24 to Cys-24 that renders cdk4 insensitive to inhibition by $p16^{INK4a}$—expanded the mass of physiologically functional β cells (19, 21). Moreover, islet-specific rescue of *cdk4* disruption prevents diabetes (18). These pathways appear to be conserved in humans, as adenoviral-mediated cyclin D1 overexpression greatly induced proliferation of human adult islets (6).

Since D-type cyclin/cdk4 complex activity regulates islet growth, we investigated whether cyclin D1 or D2 contributed

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to normal islet development, adult growth, and function. Here, we show that cyclin D2 is the main D-type cyclin expressed in islets from adult C57BL/6 × 129 mice that promotes lifelong β-cell growth. Although cyclin D2 is absolutely required for β-cell growth immediately after birth of backcrossed C57BL/6 mice (13), our results on a mixed genetic background reveal a relaxed requirement for cyclin D2 and the ability of cyclin D1 to support sufficient β-cell growth and retard the progression to diabetes until 9 to 12 months of age.

MATERIALS AND METHODS

Abbreviations: CDK, cyclin-dependent kinase; Rb, retinoblastoma-associated protein; IGF, insulin-like growth factor.

Mice. Cyclin D1-, D2-, and D3-deficient mice have been described previously (23–25), were maintained on an identical mixed genetic background (C57BL/6 × 129Sv), and were genotyped as described previously. Single knockout mice were interbred, yielding double heterozygous mice. Double heterozygotes were then intercrossed with single or double heterozygotes to yield the main genotypes of this study: wild-type, *cyclin D2*$^{-/-}$, *cyclin D1*$^{+/-}$, and *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice. Mice were maintained at a nonbarrier animal facility in the Harvard School of Public Health and the Dana Farber Cancer Institute and fed Mouse Diet 5020 9F (PMI Nutrition International, Richmond, Indiana) (9% fat calculated by weight, 21.6% fat calculated by kilocalories). Genotyping of animals was done by PCR, as described previously. Male mice were used to characterize glucose homeostasis phenotypes and pancreatic pathology. Random-fed glucose and insulin measurements were performed as previously described. Intraperitoneal glucose tolerance tests were performed on mice fasted for 15 to 16 h with 2 g D-glucose per kg of body weight as described previously (16). Intraperitoneal insulin tolerance tests were performed on fed mice with 1.5 U/kg, similar to previously described methods (16).

Statistics. All results are reported as means ± standard errors of the means (SEM) for equivalent groups. Results were compared with independent *t* tests (unpaired and two-tailed) reported as *P* values.

Reverse transcriptase PCR (RT-PCR). Islets were isolated from 6-week-old male wild-type mice with Collagenase P (Liberase RI; Roche Diagnostics Corporation, Indianapolis, Indiana) digestion, and total RNA was extracted with Trizol (GIBCO BRL; Life Technologies Inc., Grand Island, New York) and RNeasy (QIAGEN Inc., Valencia, California) columns. cDNA synthesis was performed using a RETROscript kit (Ambion Inc., Austin, Texas), and then real-time quantitative dual fluorescently labeled FRET PCR (50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 seconds, 60°C for 1 min) was performed with an ABI 7900 real-time PCR thermal cycler (Applied Biosystems., Foster City, California) to amplify samples in triplicate for the Cyclin D1, Cyclin D2, and Cyclin D3 genes. Primers were as follows: Cyclin D1 forward, TCCGC AAGCATGCACAGA; Cyclin D1 probe, 6-carboxyfluorescein (FAM)–CTTTG TGGCCCTCTGTGCCACAGA–6-carboxytetramethylrhodamine (TAMRA); Cyclin D1 reverse, GGTGGGTTGGAAATGAACTTCA; Cyclin D2 forward, GCTCTGTGCGCTACCGACTT; Cyclin D2 probe, FAM-AAGTTTGCCATG TACCCGCCATCG-TAMRA; Cyclin D2 reverse, CACGCTTCCAGTTGCAA TCA; Cyclin D3 forward, TGATTGCGCACGACTTCCT; Cyclin D3 probe, FAM-TGATTCTGCACCGCCTGTCTCTGC-TAMRA; Cyclin D3 reverse, CA AAGCCTGCCGGTCACT; cyclophilin forward, CAGACGCCACTGTCGC TTT; cyclophilin probe, FAM-CCTACACCCGGGCGCAGCTG-TAMRA; cyclophilin reverse, TGTCTTTGGAACTTTGTCTGCAA. A standard curve was generated for each primer-probe set by quantifying PCR product with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware) and performing an additional round of amplification on diluted samples across a wide range. For each gene, six replicate gene expression values were measured using equal amounts of wild-type islet cDNA and were reported as mean ± SEM. To compare D-type cyclin mRNA expression between *cyclin D2*$^{-/-}$ mice and wild-type mice, islets were isolated from four animals, RNA was extracted, and cDNA was made as described above, followed by triplicate reactions for cyclin D1, D2, and D3 as well as cyclophilin as a control. Relative gene product amounts were reported as means ± SEM for four animals for each gene expressed as percent wild-type gene expression compared to cyclophilin.

Immunohistochemistry and islet morphometry. Immunohistochemical localization of antigens and double-labeled immunohistochemistry were performed similarly to previously described methods (16). Pancreas samples were dissected from fed mice and fixed with 4% paraformaldehyde/phosphate-buffered saline solution overnight. Five-micrometer longitudinal sections of paraffin blocks were rehydrated with xylene followed by decreasing concentrations of ethanol, microwaved in 0.01 M sodium citrate (pH 6.0) for 20 min, and permeabilized with 1% Triton X-100 in phosphate-buffered saline prior to primary antisera incubation. Guinea pig anti-insulin, rabbit anti-glucagon antibodies (Zymed Laboratories Inc., South San Francisco, California), mouse anti-cyclin D2 (MS221-P, Neomarkers Inc., California), and rat anti-BrdU (BU1/75, Accurate Chemical, Westbury, NY) were used as primary antibodies. Secondary antibodies were labeled with Cy2 or Cy3 (Jackson ImmunoResearch Laboratories Inc., West Grove, Pennsylvania). Nuclear staining was performed on some sections with DAPI (4′,6′diamidino-2-phenylindole) (Molecular Probes, Eugene, Oregon) when appropriate. β-Cell area was measured by acquiring images with a Zeiss Axiovert microscope or with a Zeiss Axioskop 2 plus mot (Carl Zeiss MicroImaging, Thornwood, New York) and captured with a Hamamatsu Orca or Orca ER digital camera at ×10 from at least 20 total adjacent nonoverlapping images of two insulin-stained sections at least 300 microns apart. To measure β-cell area, islet size, and islet density, at least four pancreata were photographed: 3-month-old wild-type ($n = 4$) pancreata, 200 total islets in 137 fields; 3-month-old *cyclin D2*$^{-/-}$ mice ($n = 5$), 153 total islets in 142 fields; 3-month-old *cyclin D1*$^{+/-}$ mice ($n = 4$), 270 total islets in 223 fields; 3-month-old *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice ($n = 5$), 239 total islets in 195 fields; 9- to 12-month-old wild-type ($n = 5$) pancreata, 470 total islets in 232 fields; 9- to 12-month-old *cyclin D2*$^{-/-}$ mice ($n = 11$), 711 total islets in 498 fields. Images were analyzed by measuring β-cell area of all visible multicell islets with Open Lab software similarly to previously published studies (16). Results of β-cell quantification were expressed as the percentage of the total surveyed area containing cells positive for insulin. Islet density and size were calculated from captured insulin-stained images. Results from *cyclin D2*$^{-/-}$ mice and *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice were compared with independent *t* tests (unpaired two-tailed) and reported as *P* values. β-Cell to α-cell quantification was performed on insulin- and glucagon-stained sections from 9- to 12-month-old wild-type and *cyclin D2*$^{-/-}$ mice. At least 20 random islet sets of images per mouse, four mice per genotype, were acquired with a 20× objective and analyzed by totaling the sum area of insulin or glucagon content for each mouse. Results were reported as means ± SEM and were compared with independent *t* tests (unpaired two-tailed) expressed as a *P* value.

Neonatal islet morphometry studies were performed similarly to adult studies, with insulin-immunostained pancreatic sections isolated at postnatal day 5 with at least two cross-sectional pictures taken from five animals per genotype. Cross-sectional β-cell and pancreatic area were calculated, and β-cell area was reported as a percentage of total pancreatic area.

β-Cell proliferation studies were performed by injecting 16-day- and 3-month-old mice with BrdU (Roche) (100 μg/g of body weight) 6 h prior to sacrifice. Triple-label DAPI/insulin/BrdU immunohistochemistry images were acquired from rehydrated paraformaldehyde-fixed paraffin-embedded sections. Images of each islet per section were acquired at ×20 or ×40. BrdU-positive β-cell ratios were calculated as the means ± SEM of BrdU-positive β cells over total β cells per section, two sections per animal, at least 1,000 nuclei per animal, three to four animals per genotype. In pancreata from *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice at 3 months only 300 to 400 nuclei per animal were typically counted, due to greatly smaller islet size.

Apoptosis analysis was performed on pancreas sections from 3- and 12-month-old mice by the terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL) method using Cy2-labeled reagents (Roche) on rehydrated and trypsin-predigested pancreas sections. Sections were subsequently stained for insulin and Cy3 secondary stained with appropriate antisera, as described above. All visible islets per pancreatic section (four sections per animal, four animals per genotype) were analyzed for the presence of TUNEL-insulin copositive cells within the islet.

RESULTS

Analysis of D-type cyclin content in islets. To determine the expression pattern of D-type cyclins in pancreatic islets, we analyzed isolated adult mouse islet mRNA content by RT-PCR. All three D-type cyclins were detected, but cyclin D2 was expressed at 10-fold-higher levels than cyclin D1, with cyclin D3 expressed at far lower levels than cyclin D2 or D1 in the basal state (Fig. 1a). Immunohistochemistry studies with specific monoclonal antibodies revealed that cyclin D2 is coexpressed with insulin within islets of adult pancreata with mostly cytoplasmic staining (Fig. 1b). These studies suggest that cyclin

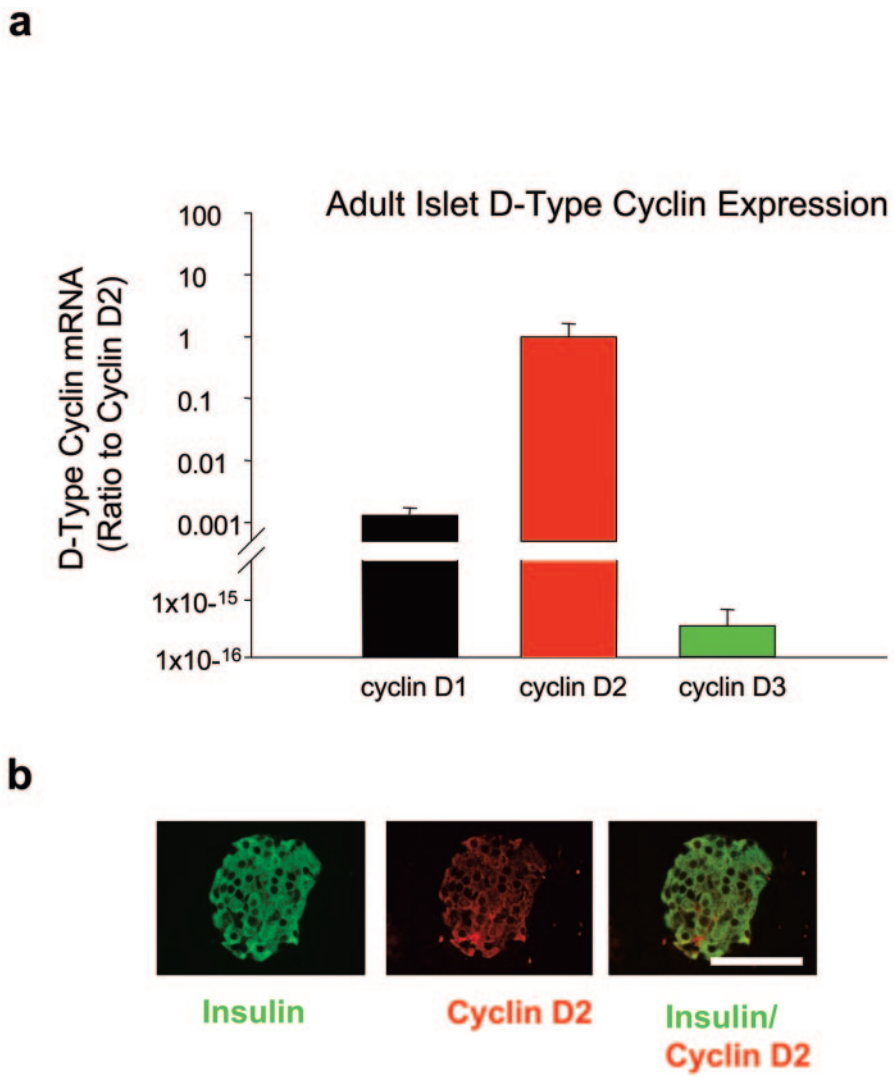
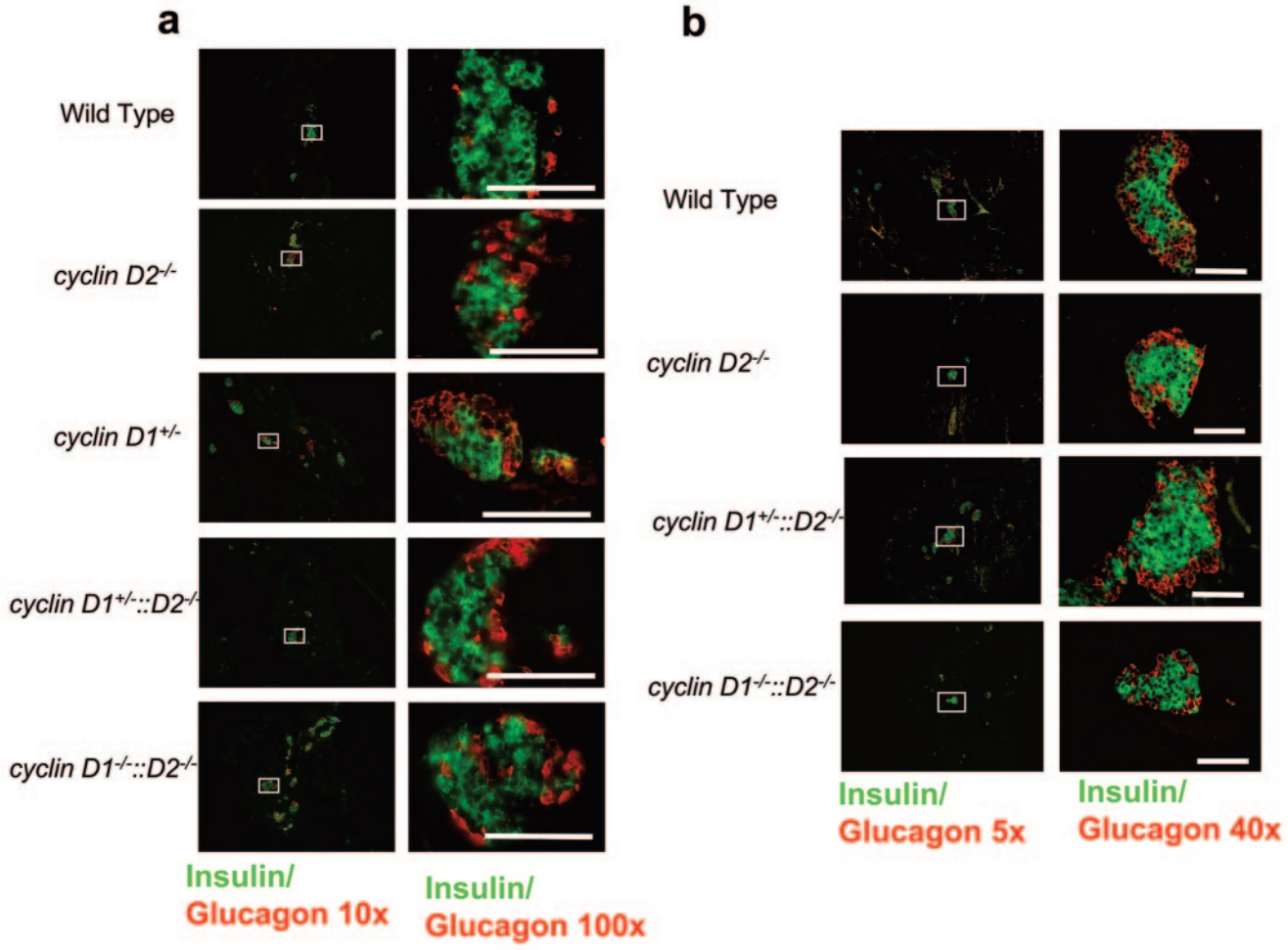


FIG. 1. D-type cyclin content analysis in mouse islets. (a) RT-PCR analysis of D-type cyclin RNA content in wild-type male mouse islets expressed as fraction of cyclin D2 expression. Results are expressed as means ± SEM for six samples per group. (b) Representative immunohistochemistry of wild-type male mouse pancreas section stained with guinea pig anti-insulin (green) and mouse anti-cyclin D2 (red) antisera. Scale bar: 100 μm.

D2 in particular could play some important role in islet function, but do not rule out a role for cyclins D1 and D3, as they could be induced in normal or pathological islet growth.

Islet development in compound D-type cyclin mice. To investigate the role of D-type cyclin function in islet physiology, we examined pancreas sections from compound *D-type cyclin* knockout mice at postnatal days 5 and 16. Disruption of the *cyclin D1* and/or *cyclin D2* genes had no effect upon the number or size of islets. Moreover, compared to wild-type mice, *cyclin D2*$^{-/-}$ mice, *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice, and *cyclin D1*$^{-/-}$ *D2*$^{-/-}$ mice had islets of equivalent sizes and distributions at 5 and 16 days with normal-appearing β-cell and α-cell morphology (Fig. 2a). Morphometric analysis of pancreata—measured by β-cell area normalized to total pancreas area—confirmed that newborn (P5) *cyclin D2*$^{-/-}$ mice and *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice have β-cell area that is equal to *cyclin D1*$^{+/-}$ mice or wild-type controls (Fig. 2b, c). Finally, we examined mice with complete absence of cyclins D1 and D2 (*cyclin D1*$^{-/-}$ *D2*$^{-/-}$ mice), which have severe central nervous system developmental abnormalities causing early neonatal death (in comparison to the normal life span of *cyclin D1*$^{-/-}$ mice, *cyclin D2*$^{-/-}$ mice, or *cyclin D3*$^{-/-}$ mice) (5). Although most *cyclin D1*$^{-/-}$ *D2*$^{-/-}$ mice die before or soon after birth, the few that lived to 16 days had euglycemic random-fed blood glucose values. Given the lethal phenotype of *cyclin D1*$^{-/-}$ *D2*$^{-/-}$ mice, it is possible that cyclins D1 and D2 could have some role in neonatal β-cell function. Nonetheless, our data suggest that cyclins D1 and D2 are not absolutely required for embryonic β-cell development.

Body composition and glucose homeostasis in cyclin D2$^{-/-}$ mice. At 3 months, *cyclin D2*$^{-/-}$ mice had normal body weights and normal fasting and fed blood glucose values (Table 1 and Table 2). However, 3-month-old *cyclin D2*$^{-/-}$ mice were glucose intolerant following intraperitoneal glucose challenge



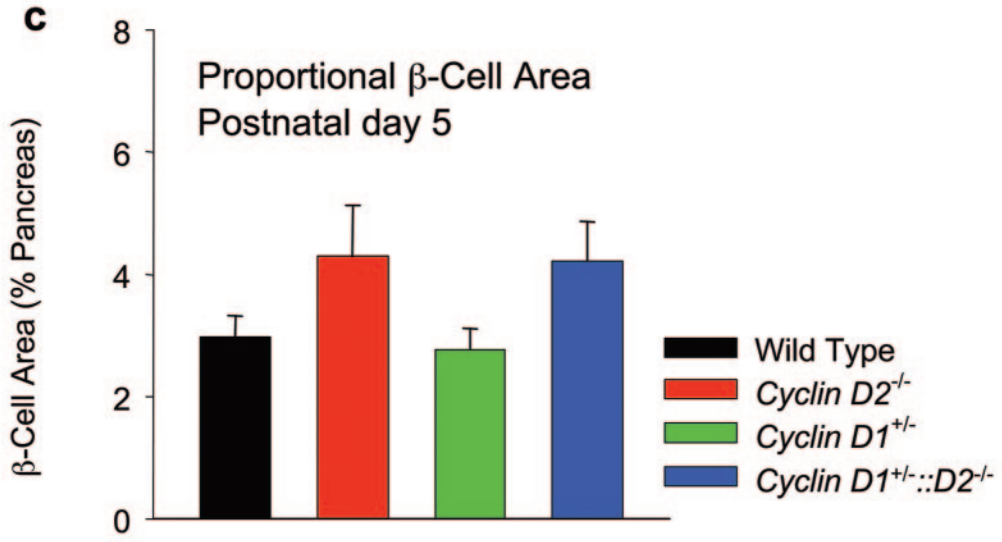


FIG. 2. Neonatal islet histology and morphometric analysis. (a) Representative islet histology of pancreas sections from 5-day-old wild-type, *cyclin D2*$^{-/-}$, *cyclin D1*$^{+/-}$, *cyclin D1*$^{+/-}$ *D2*$^{-/-}$, and *cyclin D1*$^{-/-}$ *D2*$^{+/-}$ mice. Immunostaining with antibodies against insulin (green) and glucagon (red) at ×10 (left panels) and at ×100 (right panels). Scale bars: 100 μm. (b) Sixteen-day-old wild-type, *cyclin D2*$^{-/-}$, *cyclin D1*$^{+/-}$ *D2*$^{-/-}$, and *cyclin D1*$^{-/-}$ *D2*$^{-/-}$ mice. Immunostaining with antibodies against insulin (green) and glucagon (red) at ×5 (left panels) and at ×40 (right panels). Scale bars: 100 μm. (c) Mean proportional cross-sectional β-cell area (reported as percentage of total pancreas area) of wild-type, *cyclin D2*$^{-/-}$, *cyclin D1*$^{+/-}$, and *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice at postnatal day 5.

TABLE 1. Body weights of mice by genotype

Genotype	Body wt (g)			
	3 mo	*P*	9–12 mo	*P*
Wild type	32.7 ± 1.5		37.3 ± 3.6	
cyclin D2$^{-/-}$	29.3 ± 0.9		29.4 ± 1	<0.05
cyclin D1$^{+/-}$	33.3 ± 3.0		N.D.[a]	
cyclin D1$^{+/-}$ *D2*$^{-/-}$	23.9 ± 1.3	<0.01	Dead[a]	

[a] N.D., not done.

(Fig. 3a). At 9 to 12 months, *cyclin D2*$^{-/-}$ mice developed diabetes with fasting and random-fed hyperglycemia (Table 2); glucose tolerance tests confirmed deteriorating glucose homeostasis (Fig. 3b). By comparison, intraperitoneal insulin tolerance tests showed that young and old *cyclin D2*$^{-/-}$ mice have normal peripheral insulin sensitivity (Fig. 3c-d). By 9 to 12 months of age, *cyclin D2*$^{-/-}$ mice exhibited relative fasting and fed hypoinsulinemia compared to the small cohort (5 mice) of wild-type mice (Table 2). *cyclin D2*$^{-/-}$ mice failed to gain weight at 12 months, confirming the progression to diabetes (Table 1). Thus, cyclin D2 is important for compensatory insulin secretion needed to maintain glucose homeostasis as mice age but is not required for peripheral insulin action. In comparison to published reports of the severely diabetic *cdk4*$^{-/-}$ mice (21, 26), the mild diabetic phenotype of *cyclin D2*$^{-/-}$ mice suggested that another D-type cyclin could have an accessory role in adult postnatal islet growth in the absence of cyclin D2.

Complementary roles of cyclins D1 and D2 to promote glucose homeostasis. To determine whether cyclin D1 contributes, albeit insufficiently, to the growth of *cyclin D2*$^{-/-}$ β cells, we generated *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice and *cyclin D1*$^{-/-}$ *D2*$^{-/-}$ mice. Disruption of a single *cyclin D1* allele had little effect on glucose homeostasis: *cyclin D1*$^{+/-}$ mice were euglycemic with normal fasting and fed blood glucose despite low serum insulin values (Table 2). By contrast, *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice displayed profound abnormalities in glucose homeostasis with severe fasting and random-fed hyperglycemia and death by 4 months of age from uncontrolled diabetes despite normal fasting and random-fed serum insulin levels (Table 2). As a result, glucose tolerance was severely compromised in *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice by 3 months of age (Fig. 4a). Severe diabetes of *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice is most likely due to defective insulin secretion and not altered peripheral insulin action: *cyclin D1*$^{+/-}$ mice have normal peripheral insulin sensitivity, similar to *cyclin D2*$^{-/-}$ mice (Fig. 4b). Compared to cyclins D2 and D1, cyclin D3 appears to be less important for adult regulation of glucose homeostasis, as *cyclin D2*$^{-/-}$ *D3*$^{+/-}$ mice displayed a slow progression to diabetes that resembled *cyclin D2*$^{-/-}$ mice (data not shown).

Islet histology and β-cell replication of D-type cyclin knockout mice. Histological examination revealed that β-cell mass in *cyclin D2*$^{-/-}$ mice—estimated by β-cell area normalized to total pancreas area—was near normal at 3 months (nonsignificant decrease of 30%, *P* value 0.1) but decreased by 70% at 9 to 12 months of age ($P < 0.01$) (Fig. 5a, c). Mean islet size was slightly decreased in young *cyclin D2*$^{-/-}$ mice compared to wild-type controls but greatly decreased as *cyclin D2*$^{-/-}$ mice aged (Fig. 5d). β-Cell to α-cell area ratio was reduced by ~50% in *cyclin D2*$^{-/-}$ mice at 9 to 12 months of age compared to wild-type controls (wild type, 10.9%±1.94; *cyclin D2*$^{-/-}$, 4.9%±1.1; $P = 0.036$). Thus, our results suggest that loss of cyclin D2 results in a β-cell-specific islet growth defect.

Consistent with early onset diabetes, *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice had profoundly abnormal islet histology with very small islets with few β cells and increased proportions of glucagon-containing α cells; β-cell area and mean islet size were reduced more than 80% compared to wild-type controls (Fig. 5a, c, d). In comparison, *cyclin D1*$^{+/-}$ mice had normal islet histology, β-cell area, islet size, and islet density. Islet densities—measured by islets counted per pancreatic cross-sectional area—were equivalent in *cyclin D2*$^{-/-}$ mice, *cyclin D1*$^{+/-}$ mice, and *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice at 3 months and only slightly decreased in older *cyclin D2*$^{-/-}$ mice compared to wild-type controls (Fig. 5e). β-Cell neogenesis has been speculated to arise from differentiation and proliferation of insulin-positive pancreatic ductal cells (3). However, this pathway does not appear to be induced in the absence of D-type cyclins, as insulin-positive pancreatic ductal cells were extremely rare in adult pancreata of all genotypes.

Since cyclin D1 and D2 are expected to promote cell cycle progression, we examined β-cell proliferation by performing BrdU labeling at 16 days of life. Interestingly, pancreas sections from wild-type and cyclin *D2*$^{-/-}$ mice displayed equal numbers of insulin and BrdU copositive cells (Fig. 6a, b). By contrast, β-cell proliferation was barely detected in *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice at 16 days of age (Fig. 6a, b); when D-type cyclin activity drops below a critical level β-cell duplication is almost totally ablated. Cyclin D2-dependent proliferation effects in the pancreas appear to be limited to β cells and not the exocrine compartment: pancreatic acinar cells of wild-type, *cyclin D2*$^{-/-}$, *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice contain equivalent

TABLE 2. Characteristics of mice by age group

Genotype	Result for mice in indicated age group															
	Fasting blood glucose (mg/dl)				Fed blood glucose (mg/dl)				Fasting serum insulin (pg/ml)				Fed serum insulin (pg/ml)			
	3 mo	*P*	9–12 mo	*P*	3 mo	*P*	9–12 mo	*P*	3 mo	*P*	9–12 mo	*P*	3 mo	*P*	9–12 mo	*P*
Wild type	89 ± 10		136 ± 11		139 ± 6		155 ± 15		371 ± 218		2,068 ± 1,074		1,131 ± 638		4,141 ± 1788	
cyclin D2$^{-/-}$	100 ± 10		171 ± 19		143 ± 7		262 ± 27	<0.05	≤156		213 ± 78	<0.05	1,935 ± 638		559 ± 152	<0.01
cyclin D1$^{+/-}$	71 ± 10		N.D.[a]		130 ± 3		N.D.		≤156		N.D.		259 ± 167		N.D.	
cyclin D1$^{+/-}$ *D2*$^{-/-}$	282 ± 78	<0.05	Dead		454 ± 64	<0.01	Dead		217 ± 141		Dead		1,183 ± 571		Dead	

[a] N.D., not done.

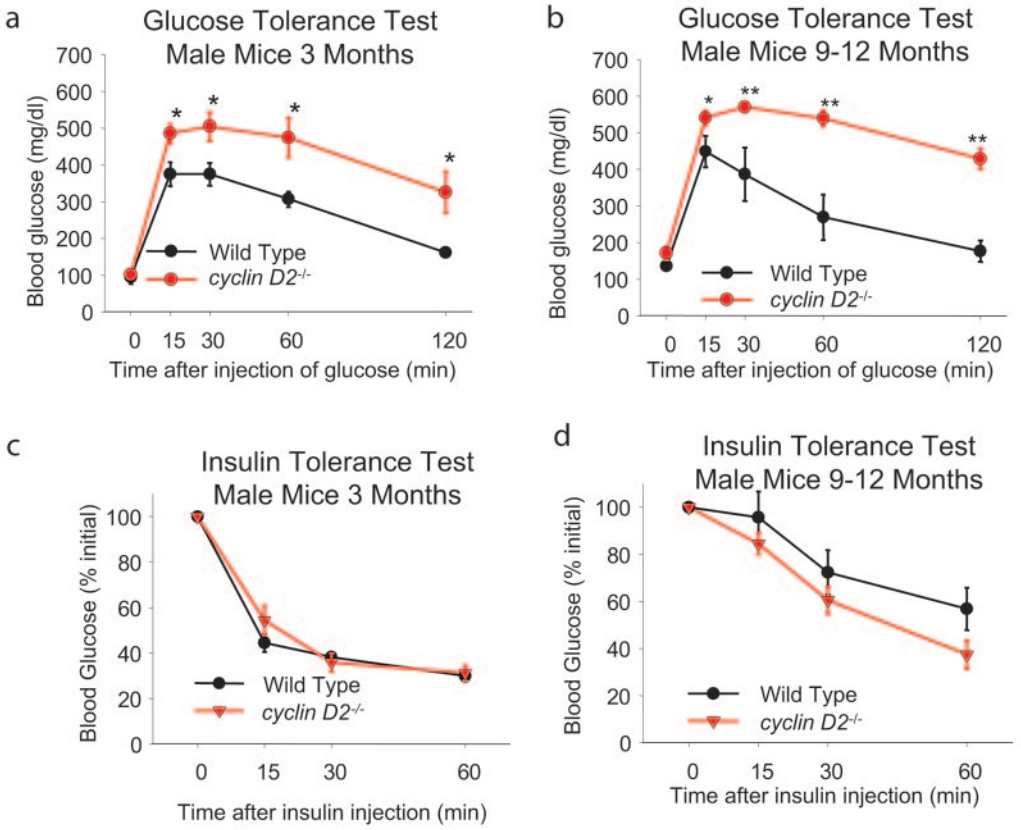


FIG. 3. Metabolic testing of male wild-type and *cyclin D2*$^{-/-}$ mice. (a to c) Glucose tolerance tests of male wild-type and *cyclin D2*$^{-/-}$ mice. Serum blood glucose measurements expressed as mg/dl of 3-month-old mice (a) and 9- to 12-month-old mice (b) after intraperitoneal injection of 2 g D-glucose/kg body weight. *, $P < 0.05$, *cyclin D2*$^{-/-}$ versus wild-type; **, $P < 0.01$, *cyclin D2*$^{-/-}$ versus wild type. (c to d) Insulin tolerance tests of male wild-type and *cyclin D2*$^{-/-}$ mice. (c) Serum blood glucose measurements expressed as means ± SEM of percentages of initial blood glucose values of 3-month-old (c) or 9- to 12-month-old (d) male wild-type and *cyclin D2*$^{-/-}$ mice after intraperitoneal injection of 1.5 U/kg human regular insulin. Results represent at least 10 mice per group, except wild-type mice at 9 to 12 months ($n = 5$).

rates of BrdU-positive cells at 16 days (Fig. 6a). By 3 months β-cell proliferation was extremely low in *cyclin D2*$^{-/-}$ mice in comparison to wild-type mice or *cyclin D1*$^{+/-}$ mice and not detected at all in *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice (Fig. 6c, d). Thus, cyclin D2 is essential for adult β-cell growth, and cyclin D1 plays a critical accessory role to promote early postnatal β-cell replication.

While proliferation pathways seem to be highly dependent on cyclins D2 and D1, islet antiapoptosis pathways could also be influenced by D-type cyclin function. To examine apoptosis we performed TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling) staining of pancreata from various genotypes in young and old mice. TUNEL-positive β cells per islet were very rare but present at roughly equivalent rates in wild-type, *cyclin D2*$^{-/-}$, and *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice at 3 months (Table 3) and wild-type and *cyclin D2*$^{-/-}$ mice at 9 to 12 months (Table 3). Thus, changes in apoptosis are unlikely to contribute to the loss of β-cell mass in 9- to 12-month-old cyclin D2$^{-/-}$ mice.

D-type cyclin gene expression in cyclin D2 knockout mice. Defective postnatal islet growth of *cyclin D2*$^{-/-}$ mice would suggest that D-type cyclins have specific and noninterchangeable roles to promote islet growth, with *cyclin D2* islet gene expression regulated in unique ways compared to cyclins D1 and D3. To test this hypothesis we performed D-type cyclin gene expression analysis in islets from wild-type and *cyclin D2*$^{-/-}$ mice. *cyclin D1* and *D3* mRNA expression failed to increase in islets from *cyclin D2*$^{-/-}$ mice above the level expressed in islets from wild-type controls (Fig. 7). Therefore, islet D-type cyclin expression appears to be overlapping but nonredundant, with islet cyclin D2 mRNA expression regulated in unique ways compared to cyclins D1 and D3.

DISCUSSION

Cyclin D2 is essential for normal postnatal islet growth and glucose homeostasis throughout adult life. This requirement was best revealed by *cyclin D2*$^{-/-}$ mice, which progress to diabetes due to altered β-cell replication and inadequate β-cell mass. Previous studies of *cyclin D2*$^{-/-}$ mice on a C57BL/6 genetic background indicated an absolute requirement for cyclin D2 after birth (13). However, our studies of *cyclin D2*$^{-/-}$ mice on a C57BL/6 sv129 genetic background indicate that cyclin D1 partially compensates for the absence of cyclin D2: *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice have a much more severe islet growth deficiency that causes death from uncontrolled diabetes by 4 months of age. Cyclin D3 does not appear to be nearly as important as cyclins D2 and D1 to promote islet function, as

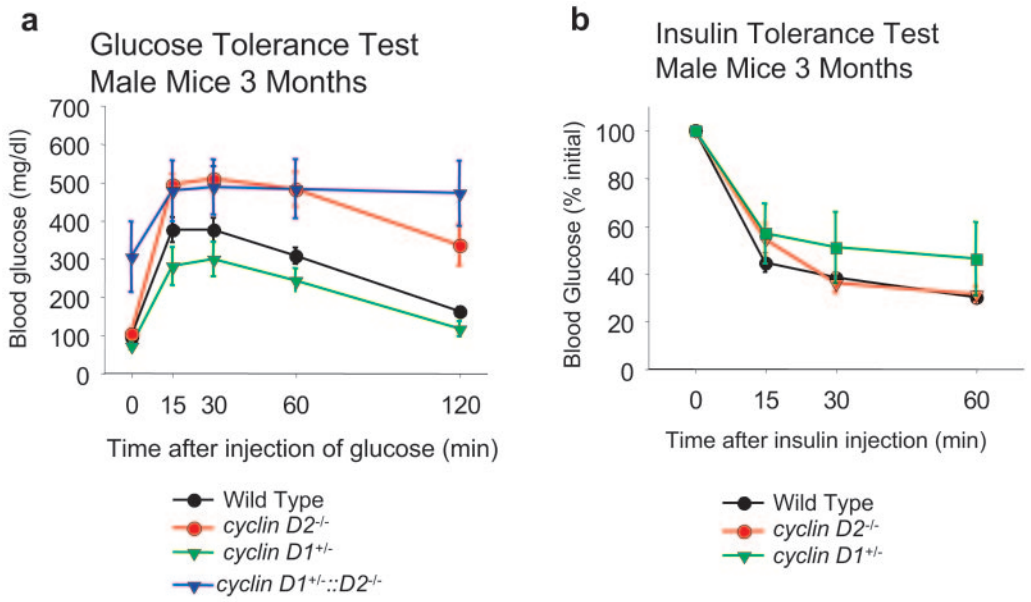


FIG. 4. Metabolic testing of *cyclin D1*$^{+/-}$ *D2*$^{+/-}$ intercross mice. (a) Glucose tolerance tests of male wild-type, *cyclin D2*$^{-/-}$, *cyclin D1*$^{+/-}$, and *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice at 3 months of age. Serum blood glucose measurements are expressed as mg/dl after intraperitoneal injection of 2 g D-glucose/kg body weight. (b) Insulin tolerance tests of male wild-type, *cyclin D2*$^{-/-}$, and *cyclin D1*$^{+/-}$ mice at 3 months of age. Serum blood glucose measurements expressed as means ± SEM of percentages of initial blood glucose values after intraperitoneal injection of 1.5 U/kg human regular insulin. Results represent at least 10 mice per group, except *cyclin D1*$^{+/-}$ mice ($n = 5$).

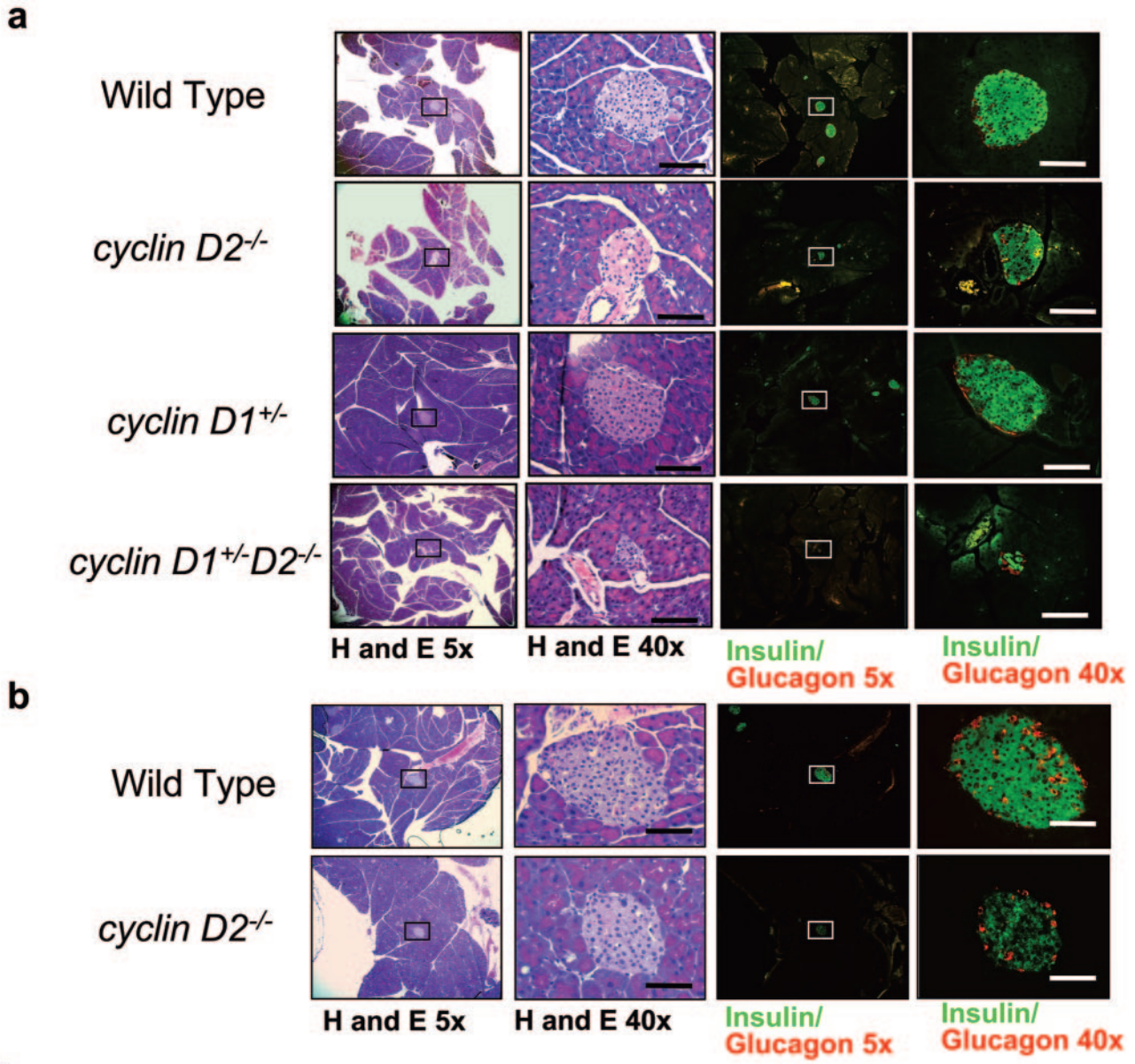
superimposed disruption of one *cyclin D3* allele does not worsen the diabetic phenotype of *cyclin D2*$^{-/-}$ mice.

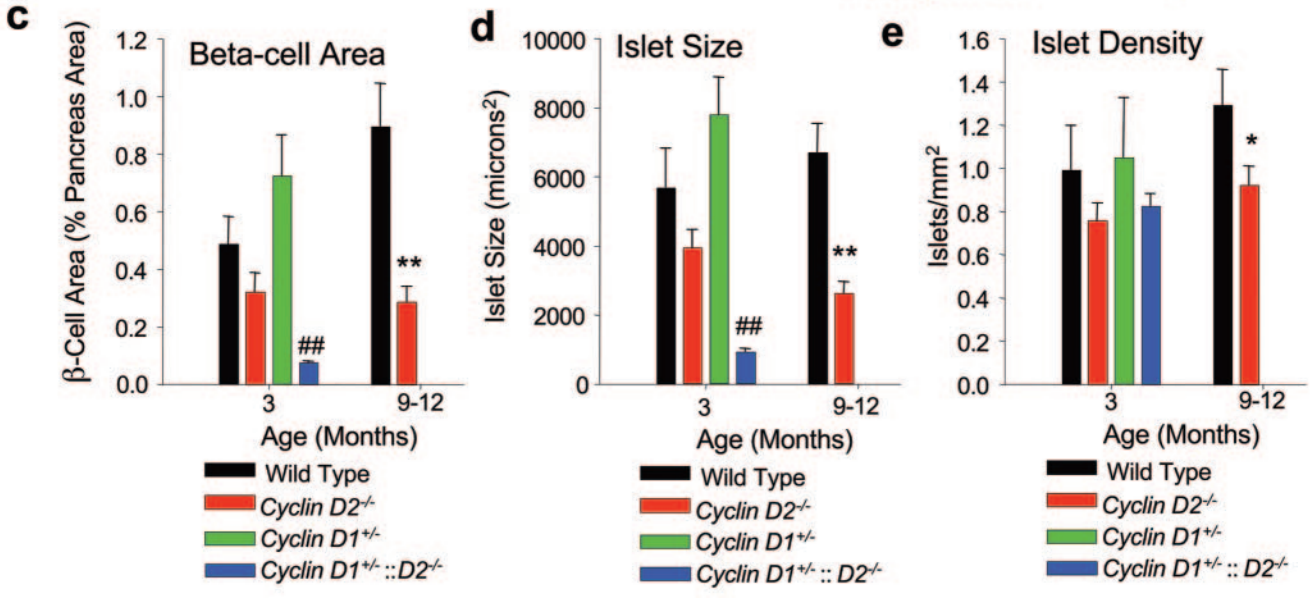
Cyclins D2 and D1 are not essential for neonatal islet development, consistent with normal development of most other tissues in *cyclin D1*$^{-/-}$ *D2*$^{-/-}$ mice (5). Cyclin D3 and/or E-type cyclins apparently compensate for the absence of cyclins D2 and D1 during β-cell development. Considerable evidence suggests that D-type and E-type cyclins have overlapping roles in development. Without all three D-type cyclins or without E type cyclins embryos develop normally, although specific fatal defects occur in cardiovascular and hematological development or placental development (12, 15). Moreover, genetic substitution of *cyclin E1* in the *cyclin D1* locus completely rescues the somatic growth and other defects characteristic of *cyclin D1*$^{-/-}$ mice (10). Similarly, disruption of the cyclin E/cdk2 inhibitor p27^{KIP1} rescues the phenotypes of *cyclin D1*$^{-/-}$ mice (11). Thus, the combined effects of D-type and E-type cyclins could promote cell cycle progression of embryonic β-cell precursors, as is the case for most somatic tissues.

Cyclin D2 appears to take on an important role in islet growth as our male mice age. *cyclin D2*$^{-/-}$ mice have normal β-cell replication at 16 days of life, but β-cell replication is extremely low by 3 months when β-cell mass begins to decrease relative to wild-type mice. Cyclin D1 and Cyclin D3 appears to be sufficient for β-cell growth in middle-aged *cyclin D2*$^{-/-}$ mice. However, *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice display profound defects in glucose homeostasis, owing to impaired islet growth consistent with undetectable adult β-cell proliferation. Superimposed *cyclin D1* haploinsufficiency on *cyclin D2* knockout mice apparently drops islet D-type cyclin activity below a critical threshold that reduces adult β-cell proliferation. As mice age cyclin D2 assumes an essential role to promote β-cell replication, and islet mass of adult *cyclin D2*$^{-/-}$ mice fails to grow and expand to compensate for increased peripheral insulin resistance. Importantly, our studies cannot differentiate the source of adult β-cell growth. BrdU-positive β cells could represent β-cell replication, as recently reported by Melton and colleagues (7). Alternatively, β-cell neogenesis might also occur, as suggested previous studies of our group and others (3, 17).

Georgia and Bhushan recently reported that *cyclin D2*$^{-/-}$ mice on a C57BL/6 genetic background develop severe diabe-

FIG. 5. Islet histology and morphometric analysis of adult wild-type, *cyclin D2*$^{-/-}$, *cyclin D1*$^{+/-}$, and *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice. (a) Representative islet histology from pancreas sections from 3-month-old male mice. Hematoxylin and eosin (H&E) staining at ×5 (furthest left panels) and at ×40 (second from left panels). Immunostaining with antibodies against insulin (green) and glucagon (red) at ×5 (second from right panels) and at ×40 (furthest right panels). Scale bars: 100 μm. (b) Representative islet histology from pancreas sections from 9- to 12-month-old male mice. Hematoxylin and eosin (H&E) staining at ×5 (furthest left panels) and at ×40 (second from left panels). Immunostaining with antibodies against insulin (green) and glucagon (red) at ×5 (second from right panels) and at ×40 (furthest right panels). Scale bars: 100 μm. (c to e) Islet morphometric studies of wild-type, *cyclin D2*$^{-/-}$, *cyclin D1*$^{+/-}$, and *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice at 3 months and wild-type and *cyclin D2*$^{-/-}$ mice at 9 to 12 months, with at least 4 to 5 mice per group analyzed. (*c*) Mean cross-sectional β-cell area, reported as percentage of total pancreas area. **, $P < 0.01$, *cyclin D2*$^{-/-}$ versus wild-type. (d) Islet size calculated by mean cross-sectional area of multicelled islets reported as microns ×10^3/islet. **, $P < 0.01$, *cyclin D2*$^{-/-}$ versus wild type. (e) Islet density calculated reported as islets per mm^2. *, $P < 0.05$, *cyclin D2*$^{-/-}$ versus wild type.


a
Wild Type
cyclin D2-/-
cyclin D1+/-
cyclin D1+/-D2-/-
H and E 5x
H and E 40x
Insulin/ Glucagon 5x
Insulin/ Glucagon 40x
b
Wild Type
cyclin D2-/-
H and E 5x
H and E 40x
Insulin/ Glucagon 5x
Insulin/ Glucagon 40x


c
Beta-cell Area
β-Cell Area (% Pancreas Area)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
##
**
3
9-12
Age (Months)
Wild Type
Cyclin D2-/-
Cyclin D1+/-
Cyclin D1+/- ::D2-/-
d
Islet Size
Islet Size (microns2)
10000
8000
6000
4000
2000
0
##
**
3
9-12
Age (Months)
Wild Type
Cyclin D2-/-
Cyclin D1+/-
Cyclin D1+/- :: D2-/-
e
Islet Density
Islets/mm2
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
3
9-12
Age (Months)
Wild Type
Cyclin D2-/-
Cyclin D1+/-
Cyclin D1+/- :: D2-/-

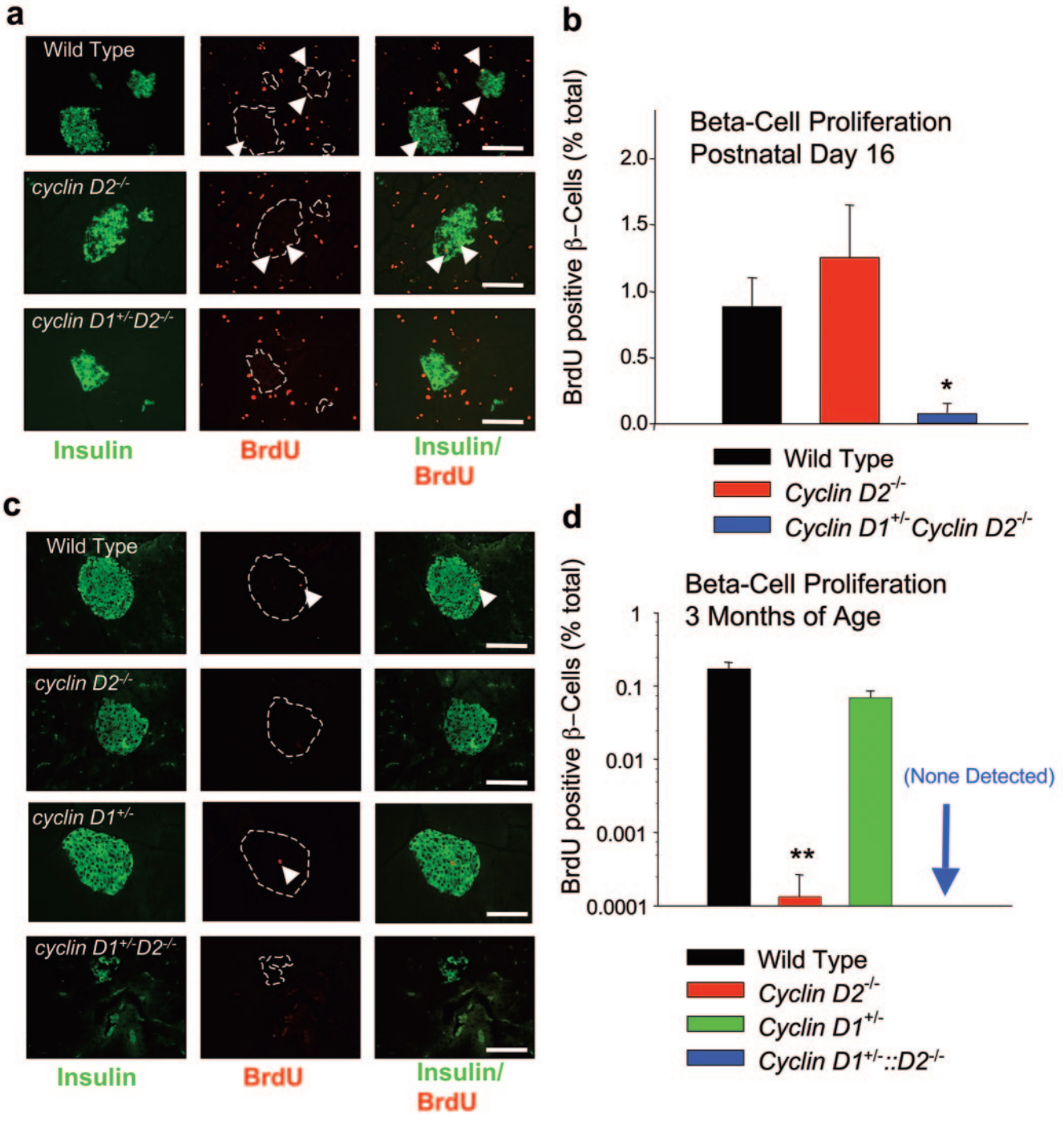


FIG. 6. Neonatal and adult islet proliferation analysis of wild-type, *cyclin D2*$^{-/-}$, *cyclin D1*$^{+/-}$, and *cyclin D1*$^{+/-}$ *D2*$^{+/-}$ mice. (a) Representative histologic islet proliferation analysis of pancreas sections from 16-day-old male mice stained with insulin (furthest left panels), BrdU (with islets outlined) (middle panels), and insulin/BrdU images merged (right panels). Proliferating β cells noted by white triangles. Images photographed at ×40, scale bars: 100 μm. (b) Percent BrdU incorporation in β cells measured at 16 days of life per genotype. **, $P < 0.01$, *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ versus cyclin *D2*$^{-/-}$ or wild type. Results are expressed as the means ± SEM fraction of BrdU/insulin copositive β cells compared to total β cells. (c) BrdU incorporation in β cells at 3 months of age per genotype. **, $P < 0.01$, cyclin *D2*$^{-/-}$ versus wild type. Results are expressed as the means ± SEM fraction of BrdU/insulin-copositive β cells compared to total β cells. (d) Representative histologic islet proliferation analysis of pancreas sections from 3-month-old male mice stained with insulin (furthest left panels), BrdU (with islets outlined) (middle panels), and insulin/BrdU images merged (right panels).

TABLE 3. TUNEL results

Genotype	No. of TUNEL-positive β-cell nuclei per islet	
	3 mo	9–12 mo
Wild type	0.0034 ± 0.0007	0.0018 ± 0.0012
cyclin D2$^{-/-}$	0.0022 ± 0.0021	0.0024 ± 0.0024
cyclin D1$^{+/-}$ *D2*$^{-/-}$	0.0020 ± 0.0020	Dead

tes by 12 weeks due to defective β-cell growth that was apparent almost immediately after birth: β-cell proliferation at 4 days of life was undetectable, and β-cell mass was decreased at 14 days of life (13). Based on their results the authors conclude that cyclin D2 is essential for normal postnatal β-cell growth. While cyclin D2 is clearly an important element in postnatal β-cell expansion, our results show that other mechanisms can compensate for the absence of cyclin D2. *cyclin D2*$^{-/-}$ mice on a C57BL/6 sv129 genetic background have normal early postnatal β-cell proliferation which ceases by 3 months of life, causing eventual diabetes. Our findings of intact β-cell proliferation in 16-day-old *cyclin D2*$^{-/-}$ mice but ablated proliferation in *cyclin D1*$^{+/-}$ *D2*$^{-/-}$ mice show that cyclin D1 contributes to β-cell expansion in C57BL/6 sv129 genetic background mice. Although all three D-type cyclins are expressed in adult islets, there is no compensatory up regulation of cyclins D1 and D3 in *cyclin D2*$^{-/-}$ mice. Thus, our studies support the unique role of cyclin D2 to promote β-cell proliferation throughout adulthood and reveal that cyclin D1 signaling can compensate in *cyclin D2*$^{-/-}$ mice.

In summary, our results point to the existence of a highly regulated postnatal islet growth pathway that integrates mitogenic stimuli with D-type cyclin/cyclin-dependent kinase function in islets, allowing metabolic requirements to be closely matched to insulin secretion capacity over a wide dynamic range. Outstanding issues include how cyclin D2 is regulated in islets, although recent evidence suggests that growth hormone-mediated islet growth may involve STAT5 regulation of the *cyclin D2* promoter (9). Future studies will be aimed at identification of the upstream and downstream components of cyclin D2-dependent islet growth. Efforts to better understand the regulation of these pathways could lead to a better understanding of the regulation of islet growth and function in general, which will hopefully result in the development of novel classes of treatments for diabetes patients.

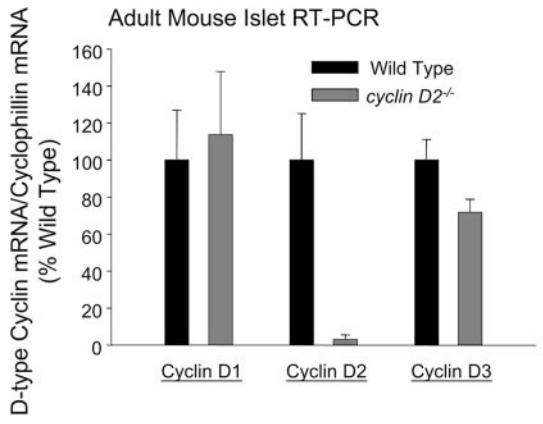


FIG. 7. D-type cyclin mRNA expression in pancreatic islets from *cyclin D2*$^{-/-}$ mice and controls. TaqMan RT-PCR of cyclin D1, D2, and D3 with islet mRNA from male *cyclin D2*$^{-/-}$ mice ($n = 4$) and wild-type controls ($n = 4$) at 6 weeks of age. Data are normalized to cyclophilin gene expression and expressed as means ± SEM.

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REFERENCES

1. **Bell, G. I., and K. S. Polonsky.** 2001. Diabetes mellitus and genetically programmed defects in beta-cell function. Nature **414:**788–791.
2. **Bonner-Weir, S.** 2001. Beta-cell turnover: its assessment and implications. Diabetes **50**(Suppl. 1)**:**S20–S24.
3. **Bonner-Weir, S., E. Toschi, A. Inada, P. Reitz, S. Y. Fonseca, T. Aye, and A. Sharma.** 2004. The pancreatic ductal epithelium serves as a potential pool of progenitor cells. Pediatr. Diabetes **5**(Suppl. 2)**:**16–22.
4. **Butler, A. E., J. Janson, S. Bonner-Weir, R. Ritzel, R. A. Rizza, and P. C. Butler.** 2003. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes **52:**102–110.
5. **Ciemerych, M. A., A. M. Kenney, E. Sicinska, I. Kalaszczynska, R. T. Bronson, D. H. Rowitch, H. Gardner, and P. Sicinski.** 2002. Development of mice expressing a single D-type cyclin. Genes Dev. **16:**3277–3289.
6. **Cozar-Castellano, I., K. K. Takane, R. Bottino, A. N. Balamurugan, and A. F. Stewart.** 2004. Induction of beta-cell proliferation and retinoblastoma protein phosphorylation in rat and human islets using adenovirus-mediated transfer of cyclin-dependent kinase-4 and cyclin D1. Diabetes **53:**149–159.
7. **Dor, Y., J. Brown, O. I. Martinez, and D. A. Melton.** 2004. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. Nature **429:**41–46.
8. **Fantl, V., G. Stamp, A. Andrews, I. Rosewell, and C. Dickson.** 1995. Mice lacking cyclin D1 are small and show defects in eye and mammary gland development. Genes Dev. **9:**2364–2372.
9. **Friedrichsen, B. N., H. E. Richter, J. A. Hansen, C. J. Rhodes, J. H. Nielsen, N. Billestrup, and A. Moldrup.** 2003. Signal transducer and activator of transcription 5 activation is sufficient to drive transcriptional induction of cyclin D2 gene and proliferation of rat pancreatic beta-cells. Mol. Endocrinol. **17:**945–958.
10. **Geng, Y., W. Whoriskey, M. Y. Park, R. T. Bronson, R. H. Medema, T. Li, R. A. Weinberg, and P. Sicinski.** 1999. Rescue of cyclin D1 deficiency by knockin cyclin E. Cell **97:**767–777.
11. **Geng, Y., Q. Yu, E. Sicinska, M. Das, R. T. Bronson, and P. Sicinski.** 2001. Deletion of the p27Kip1 gene restores normal development in cyclin D1-deficient mice. Proc. Natl. Acad. Sci. USA **98:**194–199.
12. **Geng, Y., Q. Yu, E. Sicinska, M. Das, J. E. Schneider, S. Bhattacharya, W. M. Rideout, R. T. Bronson, H. Gardner, and P. Sicinski.** 2003. Cyclin E ablation in the mouse. Cell **114:**431–443.
13. **Georgia, S., and A. Bhushan.** 2004. Beta cell replication is the primary mechanism for maintaining postnatal beta cell mass. J. Clin. Investig. **114:** 963–968.
14. **Hennige, A. M., D. J. Burks, U. Ozcan, R. N. Kulkarni, J. Ye, S. Park, M. Schubert, T. L. Fisher, M. A. Dow, R. Leshan, M. Zakaria, M. Mossa-Basha, and M. F. White.** 2003. Upregulation of insulin receptor substrate-2 in pancreatic beta cells prevents diabetes. J. Clin. Investig. **112:**1521–1532.
15. **Kozar, K., M. A. Ciemerych, V. I. Rebel, H. Shigematsu, A. Zagozdzon, E. Sicinska, Y. Geng, Q. Yu, S. Bhattacharya, R. T. Bronson, K. Akashi, and P. Sicinski.** 2004. Mouse development and cell proliferation in the absence of d-cyclins. Cell **118:**477–491.
16. **Kushner, J. A., J. Ye, M. Schubert, D. J. Burks, M. A. Dow, C. L. Flint, S. Dutta, C. V. Wright, M. R. Montminy, and M. F. White.** 2002. Pdx1 restores beta cell function in Irs2 knockout mice. J. Clin. Investig. **109:**1193–1201.
17. **Lin, X., A. Taguchi, S. Park, J. A. Kushner, F. Li, Y. Li, and M. F. White.** 2004. Dysregulation of insulin receptor substrate 2 in beta cells and brain causes obesity and diabetes. J. Clin. Investig. **114:**908–916.
18. **Martin, J., S. L. Hunt, P. Dubus, R. Sotillo, F. Nehme-Pelluard, M. A. Magnuson, A. F. Parlow, M. Malumbres, S. Ortega, and M. Barbacid.** 2003. Genetic rescue of Cdk4 null mice restores pancreatic beta-cell proliferation but not homeostatic cell number. Oncogene **22:**5261–5269.

19. **Marzo, N., C. Mora, M. E. Fabregat, J. Martin, E. F. Usac, C. Franco, M. Barbacid, and R. Gomis.** 2004. Pancreatic islets from cyclin-dependent kinase 4/R24C (Cdk4) knockin mice have significantly increased beta cell mass and are physiologically functional, indicating that Cdk4 is a potential target for pancreatic beta cell mass regeneration in Type 1 diabetes. Diabetologia **47:**686–694.
20. **Mathis, D., L. Vence, and C. Benoist.** 2001. β-Cell death during progression to diabetes. Nature **414:**792–798.
21. **Rane, S. G., P. Dubus, R. V. Mettus, E. J. Galbreath, G. Boden, E. P. Reddy, and M. Barbacid.** 1999. Loss of cdk4 expression causes insulin-deficient diabetes and cdk4 activation results in β-islet cell hyperplasia. Nat. Genet. **22:**44–52.
22. **Sherr, C. J.** 2000. The Pezcoller lecture: cancer cell cycles revisited. Cancer Res. **60:**3689–3695.
23. **Sicinska, E., I. Aifantis, L. Le Cam, W. Swat, C. Borowski, Q. Yu, A. A. Ferrando, S. D. Levin, Y. Geng, H. von Boehmer, and P. Sicinski.** 2003. Requirement for cyclin D3 in lymphocyte development and T cell leukemias. Cancer Cell **4:**451–461.
24. **Sicinski, P., J. L. Donaher, Y. Geng, S. B. Parker, H. Gardner, M. Y. Park, R. L. Robker, J. S. Richards, L. K. McGinnis, J. D. Biggers, J. J. Eppig, R. T. Bronson, S. J. Elledge, and R. A. Weinberg.** 1996. Cyclin D2 is an FSH-responsive gene involved in gonadal cell proliferation and oncogenesis. Nature **384:**470–474.
25. **Sicinski, P., J. L. Donaher, S. B. Parker, T. Li, A. Fazeli, H. Gardner, S. Z. Haslam, R. T. Bronson, S. J. Elledge, and R. A. Weinberg.** 1995. Cyclin D1 provides a link between development and oncogenesis in the retina and breast. Cell **82:**621–630.
26. **Tsutsui, T., B. Hesabi, D. S. Moons, P. P. Pandolfi, K. S. Hansel, A. Koff, and H. Kiyokawa.** 1999. Targeted disruption of CDK4 delays cell cycle entry with enhanced p27(Kip1) activity. Mol. Cell. Biol. **19:**7011–7019.
27. **Uchida, T., T. Nakamura, N. Hashimoto, T. Matsuda, K. Kotani, H. Sakane, Y. Kido, Y. Hayashi, K. I. Nakayama, M. F. White, and M. Kasuga.** 2005. Deletion of the p27Kip1 gene ameliorates hyperglycemia by maintaining compensatory hyperinsulinemia in diabetic mice. Nat. Med. **11**:175–182.
28. **White, M. F.** 2002. IRS proteins and the common path to diabetes. Am. J. Physiol. Endocrinol. Metab. **283:**E413–E422.
29. **Withers, D. J., D. J. Burks, H. H. Towery, S. L. Altamuro, C. L. Flint, and M. F. White.** 1999. Irs-2 coordinates Igf-1 receptor-mediated beta-cell development and peripheral insulin signalling. Nat. Genet. **23:**32–40.
30. **Withers, D. J., J. S. Gutierrez, H. Towery, D. J. Burks, J. M. Ren, S. Previs, Y. Zhang, D. Bernal, S. Pons, G. I. Shulman, S. Bonner-Weir, and M. F. White.** 1998. Disruption of IRS-2 causes type 2 diabetes in mice. Nature **391:**900–904.
31. **Yoon, K. H., S. H. Ko, J. H. Cho, J. M. Lee, Y. B. Ahn, K. H. Song, S. J. Yoo, M. I. Kang, B. Y. Cha, K. W. Lee, H. Y. Son, S. K. Kang, H. S. Kim, I. K. Lee, and S. Bonner-Weir.** 2003. Selective beta-cell loss and alpha-cell expansion in patients with type 2 diabetes mellitus in Korea. J. Clin. Endocrinol. Metab. **88:**2300–2308.