Glucose Regulates Cyclin D2 Expression in Quiescent and Replicating Pancreatic β-Cells Through Glycolysis and Calcium Channels

Seth J. Salpeter, Agnes Klochendler, Noa Weinberg-Corem, Shay Porat, Zvi Granot, A. M. James Shapiro, Mark A. Magnuson, Amir Eden, Joseph Grimsby, Benjamin Glaser, and Yuval Dor

Understanding the molecular triggers of pancreatic β-cell proliferation may facilitate the development of regenerative therapies for diabetes. Genetic studies have demonstrated an important role for cyclin D2 in β-cell proliferation and mass homeostasis, but its specific function in β-cell division and mechanism of regulation remain unclear. Here, we report that cyclin D2 is present at high levels in the nucleus of quiescent β-cells in vivo. The major regulator of cyclin D2 expression is glucose, acting via glycolysis and calcium channels in the β-cell to control cyclin D2 mRNA levels. Furthermore, cyclin D2 mRNA is down-regulated during S-G2-M phases of each β-cell division, via a mechanism that is also affected by glucose metabolism. Thus, glucose metabolism maintains high levels of nuclear cyclin D2 in quiescent β-cells and modulates the down-regulation of cyclin D2 in replicating β-cells. These data challenge the standard model for regulation of cyclin D2 during the cell division cycle and suggest cyclin D2 as a molecular link between glucose levels and β-cell replication. (Endocrinology 152: 2589–2598, 2011)
target of nuclear factor κB (14), β-catenin (15), and activator protein-1 (16), whereas cyclin D2 is induced by FSH (12), cAMP (17), Stat5 (18), and others. In the case of pancreatic β-cells, genetic analyses have established that cyclin D2 is a key factor in β-cell proliferation, during normal postnatal life in the mouse (4, 19, 20) as well as under conditions of insulin resistance (21). Furthermore, studies in β-cells under specific conditions that enhance β-cell proliferation, such as the transgenic expression of a constitutively active β-catenin mutant (22), a constitutively active serine-threonine kinase (AKT) (23), a constitutively active nuclear factor of activated T cells (NFAT) (24), glucose infusion (25), or exposure to prolatin and GH (26), have suggested that an increase in the overall levels of cyclin D2 may be responsible for cell cycle entry of quiescent β-cells. A recent article has also suggested that cyclin D2 may expand β-cell mass by preventing programmed cell death (27). Taken together, these studies demonstrate that cyclin D2 is important for β-cell expansion and suggest that its up-regulation might be a key link between extracellular mitogens and cell cycle entry.

Given the central role of cyclin D2 in the process of β-cell replication, we set out to examine the regulation of cyclin D2 expression in quiescent and replicating β-cells. Here, we show that cyclin D2 is expressed at high levels in the nucleus of almost all quiescent pancreatic β-cells. This high basal expression level is maintained by glucose metabolism and calcium signaling in β-cells, which control cyclin D2 mRNA levels. Lastly, we show that glucose metabolism also controls cyclin D2 levels during β-cell replication, causing down-regulation of cyclin D2 mRNA and protein during S-G2-M phases of the cell division cycle.

Materials and Methods

Immunofluorescence

Paraffin sections of the pancreas were prepared and stained as described previously (2). The antibodies used in this study for immunohistochemistry were: rabbit anticyclin D2 (1:1000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), mouse anti-cyclin D2 (1:100; Neomarkers, Fremont, CA), mouse antiproliferating cell nuclear antigen (PCNA) (1:500; Dako, Glostrup, Denmark), mouse anti-bromodeoxyuridine (BrdU) (1:300; Amersham/GE Healthcare, Princeton, NJ), and guinea pig antianti-insulin (1:200; Dako). Antigen retrieval was performed using citrate buffer and a pressure cooker (Biocare, Concord, CA) for all antibodies. All primary antibodies are left overnight in CasBlock (Zymed, San Francisco, CA). Secondary antibodies were purchased from Jackson ImmunoResearch (West Grove, PA), diluted 1:500 in PBS, and left for 1 h. Images were taken using a Nikon i90 C1 confocal microscope (Nikon, Melville, NY).

Cell culture

Islets were isolated as previously described (28). After extraction, islet cultures were performed in RPMI 1640, 1% serum, and with varying glucose concentrations for 20 h. Islet lysate was then prepared by adding buffer A [20 mM Tris (pH 7.5), 5 mM EDTA, 4.45 mg/ml of Na2P2O7, and 1% Nonidet P-40]. All cell culture experiments were performed at least three times with consistent results unless otherwise noted in the figure legend. Min-6 cells (passage 50–60) were cultured in DMEM 1% fetal bovine serum (FBS) at varying glucose concentration. Cadaveric human islets were obtained from Edmonton (Canada) and cultured in RPMI with 1% heat-inactivated FBS. Results were confirmed on two separate donors, each with at least n = 3.

The following chemicals and their concentrations were used: BayK8644, 60 μM (Sigma, St. Louis, MO); Verapamil, 10 or 30 μM (Sigma); cyclohexamide, 3 μM; insulin, 20 μM (Novo Nordisk, Bagsværd, Denmark); glucokinase (GCK) activator (GKA), 3 μM (Roche); and Arry-142886, 2 μM (Selleck, Houston, TX). For in vitro experiments, BayK8644, Arry-142886, and GKA were prepared in dimethylsulfoxide (DMSO).

Reverse transcription-polymerase chain reaction

The level of cyclin D2 mRNA was determined using a commercial TaqMan Probe (Applied Biosystems, Foster City, CA) and were normalized to TATA-binding protein (Applied Biosystems). GCK RT-PCR was performed using SYBR Green with the following sequences: forward, TGCTACTATGGAGACCCTGCAAT and reverse, CTTCACCAAGCTCCTATTCC at a working concentration of 10 pmol/μl. RNA of replicating islet cells was obtained by sorting green fluorescent protein (GFP) + cells from dissociated islets of transgenic cyclin B1-GFP mice (Kloechendler, A., N. Weinberg-Corem, A. Eden, and Y. Dor, unpublished observations), and values were normalized to actin with the following sequences: forward, CGCCATGATGAGACCGCCAAT and reverse, CACAGGGAGTCCTTTGAC. All experiments were performed on an Applied Biosystems 7300 Real-Time PCR system.

Western blotting

Western blottings were performed using 10% acrylamide gels and a miniprotein gel system (Bio-Rad, Hercules, CA). Primary antibodies were blotted overnight, and secondary antibodies (Dako) were hybridized for 1 h. Primary antibodies were used at a concentration of 1:1000. In addition to antibodies described in Immunofluorescence, the following antibodies were used for Western blotting: rabbit anti-p-AKT (Cell Signaling, Beverly, MA), mouse antiactin (Sigma), rabbit anti-CDK4 (Santa Cruz Biotechnology, Inc.), mouse anti-p16 (Santa Cruz Biotechnology, Inc.), rabbit anti-p18 (Santa Cruz Biotechnology, Inc.), mouse anti-p27 (Santa Cruz Biotechnology, Inc.), mouse antisorting green fluorescent protein (GFP) + cells from dissociated islets of transgenic cyclin B1-GFP mice (Kloechendler, A., N. Weinberg-Corem, A. Eden, and Y. Dor, unpublished observations), and guinea pig anti-β-catenin (Biosystems 7300 Real-Time PCR system).

Mouse strains

All mice used in these experiments were male and on ICR background. Sections of cyclin D2 heterozygous and wild-type littermates, originally described by Sicinski et al. (12), were the generous gifts of Anil Bhushan and Jake Kushner. Mice were
Results

Cyclin D2 is present in the nucleus of quiescent \( \beta \)-cells

D-type cyclin levels in some cell types decrease significantly once they enter quiescence and increase again if and when they reenter the cell cycle (9–13). We thus hypothesized that the expression pattern of cyclin D2 will identify the small subpopulation of \( \beta \)-cells that undergo a transition from quiescence to replication within postnatal islets. To test this idea, we stained paraffin sections of mouse pancreas using an antibody against cyclin D2. Surprisingly, in 1-wk-old mice, we observed a strong nuclear signal in the majority of \( \beta \)-cells (Fig. 1A), even though only 9% of \( \beta \)-cells are cycling at this age (Supplemental Fig. 1, published on The Endocrine Society’s Journals Online web site at http://endo.endojournals.org). Sections of the pancreas from 1-wk-old cyclin D2\( ^{−/−} \) mice showed no signal in \( \beta \)-cells or in the exocrine pancreas, verifying antibody specificity (Fig. 1, A and B). We then examined the expression of cyclin D2 in \( \beta \)-cells of 1-month-old mice. As shown in Fig. 1C and Supplemental Fig. 1, only approximately 7% of \( \beta \)-cells at this age stain for the general proliferation marker PCNA. However, approximately 90% of \( \beta \)-cells in such mice contain cyclin D2 in their nucleus, indicating that cyclin D2 is present in almost all quiescent \( \beta \)-cells. To validate this finding, we repeated the staining with a different cyclin D2 antibody and obtained identical results (Supplemental Fig. 2). We also examined the expression of cyclin A, a known marker of S-G\(_2\) phases of the cell cycle, to verify our ability to detect a cell cycle phase-specific expression pattern. Costaining for cyclin A and BrdU in mice injected with BrdU 2 h before killing revealed that cyclin A is present, as expected, only in the nuclei of BrdU\( ^{+} \) \( \beta \)-cells (Fig. 1D). Lastly, given the known age-related decline in \( \beta \)-cell proliferation, we examined whether cyclin D2 levels decreased with age. Using both immunohistochemistry and Western blot analysis, we found that there was no decrease in levels of cyclin D2 between 1- and 6-month-old \( \beta \)-cells (Supplemental Fig. 3, A and B). Taken together, these results indicate that in contrast to the standard model, the presence of abundant cyclin D2 in the nucleus of \( \beta \)-cells is not a marker of proliferation.

Cyclin D2 expression is controlled by glucose in mouse and human \( \beta \)-cells

Previous studies have shown that an increase in glucose yields an increase in cyclin D2 levels (25, 31). Other studies
have shown that both prolactin and human GH (26), as well as β-catenin (22), calcineurin/NFAT (24), and AKT (23), are able to up-regulate β-cell cyclin D2 levels. However, although numerous factors have been shown to increase D2 levels, it is unclear which physiological factors actually maintain the high levels of cyclin D2 expression in normal β-cells. Indeed, a factor that correlates with the down-regulation of cyclin D2 has yet to be demonstrated.

Given the central role of glucose in β-cell function and proliferation, we hypothesized that glucose is responsible for maintaining basal levels of cyclin D2 in pancreatic β-cells. First, we cultured both primary pancreatic islets and Min-6 insulinoma cells at different glucose concentrations for 20 h. Among numerous cell cycle markers examined, only the expression of cyclin D2 significantly changed in response to glucose levels in the medium (Fig. 2A). In both islets and Min-6 cells, cyclin D2 was gradually down-regulated as glucose was lowered, ultimately dropping by approximately 50% between 10 and 1 mM glucose in Min-6 cells and decreasing by 50% between 5 and 0.5 mM in isolated mouse islets.

We next examined whether human islets are subject to a similar regulation. First, we performed Western blot analysis comparing mouse and human D-type cyclin levels in islets from adults (8 months for mouse and 54 yr for human). For all cyclins, human cyclin levels were lower than mouse cyclins, yet the proteins were expressed (Fig. 2B). We then cultured cadaveric human islets at 10 and 2.5 mM glucose for 48 h and examined whether their cyclin D2 was down-regulated in low glucose. Similar to the mouse results, we found that cyclin D2 was gradually down-regulated when the glucose concentration in the medium was lowered (Fig. 2C).

To confirm the connection between glucose levels and cyclin D2 in vivo, we made use of two mouse models where pancreatic β-cells were exposed to low levels of glucose. First, we implanted insulin pumps in 1-month-old mice for 3 d. After 24 h, blood glucose dropped to approximately 50 mg/dl and was maintained at this level for 48 h. When mice were killed and pancreas was stained for cyclin D2, we found a dramatic decrease in the levels of β-cell but not acinar, cyclin D2 (Fig. 3A). To test whether this result was connected to high systemic insulin levels released from the pumps, we made use of another mouse model mirroring systemic hypoglycemia. Here, we crossed PdxCreER mice (32), expressing a tamoxifen-dependent Cre recombinase in β-cells, with mice containing a floxed allele of GCK (33). At 1 month of age, these PdxCreER; GCK lox/lox mice were injected with three doses of 8 mg of tamoxifen to delete GCK in β-cells. GCK deficiency in β-cells caused glucose levels to rise to 500–600 mg/dl, due to reduced glycolysis in β-cells and consequently reduced

FIG. 2. Cyclin D2 is down-regulated in response to low glucose in cultured mouse and human islets. A, Western blottings of cell cycle markers in Min-6 cells and mouse islets cultured in the indicated glucose concentrations. Only cyclin D2 was significantly reduced by glucose. Islets were cultured for 20 h after isolation. ANOVA yielded P < 0.01 for cyclin D2 difference in Min-6 and P < 0.05 for cyclin D2 difference in islets. B, Expression of D-type cyclins in islets from an 8-month-old mouse (M) and a 54-yr-old human (H), showing presence of all three proteins in both species. C, Reduced levels of cyclin D2 in human islets cultured for 48 h in low glucose. *, P < 0.05.
After 1 wk, mice were killed, and pancreas was stained for cyclin D2. Here, under conditions of systemic hypoinsulinemia/hyperglycemia but deficiency of glycolysis within β-cells, cyclin D2 had been dramatically down-regulated (Fig. 3B). Finally, to confirm that cyclin D2 levels were directly regulated by GCK enzyme activity, we cultured mouse islets with a small molecule GKA for 3 h (Fig. 3C) after an overnight culture in 2.5 mM glucose. Here, we found a 2-fold increase in the levels of cyclin D2, establishing that cyclin D2 expression is directly controlled by GCK activity.

These results demonstrate that systemic glucose maintains the high level of basal cyclin D2, both in vitro and in vivo. Moreover, they suggest that glucose controls cyclin D2 levels via GCK, glycolysis, and ATP production. The down-regulation of cyclin D2 in β-cells but not in acinar cells suggests that the glucose-dependent regulation of cyclin D2 is β-cell specific.

**Glucose controls cyclin D2 levels through a calcium-dependent pathway**

We next investigated the molecular pathway by which glucose controls β-cell cyclin D2 levels. Downstream of GCK and glycolysis, a major signaling pathway in β-cells involves membrane depolarization and calcium entry, leading to glucose-stimulated insulin exocytosis. Furthermore, calcium was shown to have important roles in β-cell replication (35). We therefore hypothesized that glucose may control cyclin D2 via calcium entry. To test this hypothesis, we cultured mouse islets in normal (5 mM) and low (0.5 mM) glucose for 20 h and then treated the islets with the calcium channel opener BayK8644 (60 μM) for 3 h. We then examined whether the low glucose-mediated decline in cyclin D2 was rescued by BayK8644. Although cyclin D2 was down-regulated in response to lower glucose when vehicle was added, BayK8644-treated islets maintained normal cyclin D2 despite low glucose, indicating that calcium channel opening can rescue glucose-dependent cyclin D2 down-regulation (Fig. 4A).
To further validate that calcium entry is responsible for glucose maintenance of cyclin D2 levels, we used a calcium channel blocker. When Verapamil was added to Min-6 cells (10 mM for 3 h) or to islets (30 mM for 20 h) under conditions of normal glucose, cyclin D2 was dramatically down-regulated (Fig. 4B). These results demonstrate that glucose determines the basal levels of cyclin D2 in β-cells via glycolysis, membrane depolarization, and voltage-gated calcium channels.

Lastly, we examined the possibility that paracrine insulin signaling downstream of calcium depolarization is the main regulator of cyclin D2 levels. Min-6 cells were cultured for 20 h at 10 mM glucose and 1 mM glucose, after which insulin was added to the culture for 3 h (Supplemental Fig. 4A). Although insulin caused increased phosphorylation of AKT, it did not up-regulate cyclin D2 levels in low glucose. Next, given that insulin is known to activate pERK signaling in β-cells (36), we examined whether inhibition of ERK signaling impacts cyclin D2 levels by culturing Min-6 with a the pERK inhibitor Arry-142886 (Supplemental Fig. 4B). After 3 h of culture, pERK was down-regulated, yet cyclin D2 was not affected. Taken together, these results suggest that insulin signaling is not involved in β-cell cyclin D2 regulation.

Low glucose down-regulates cyclin D2 mRNA

Conflicting reports exist in the literature as to whether cyclin D2 up-regulation in response to glucose stimulus occurs at the mRNA (31) or protein level (25). We examined the mRNA levels of cyclin D2 using quantitative real-time PCR and found a significant down-regulation in mRNA levels in both Min-6 cells and islets cultured in low glucose (Fig. 5A).

Previous reports have demonstrated that cyclin D2 protein has a short half-life of approximately 1 h in β-cells (27). To test whether glucose controls cyclin D2 protein stability, we treated islets with cyclohexamide to block protein synthesis and examined the rate of decay of cyclin D2, reflecting its degradation rate. As previously reported, cyclin D2 had a short half-life of approximately 1 h. However, the rate of decay did not differ in islets cultured in normal or low glucose (Fig. 5B). These results suggest that hypoglycemia-dependent down-regulation of cyclin D2 operates mostly at the mRNA level.

Cyclin D2 is down-regulated during S-G2-M phases of the cell division cycle via a glucose-dependent pathway

We noticed that a significant number of proliferating β-cells did not stain for cyclin D2 (Fig. 1C). To examine the dynamics of cyclin D2 during the cell division cycle in β-cells, we costained sections of pancreata from 1-month-old mice for insulin, cyclin D2, and BrdU (injected 2 or 7 h before killing). Only 30% of BrdU+ β-cells expressed cyclin D2 at 2 h and 20% at 7 h, whereas 80% of nonreplicating, BrdU− β-cells stained positive for cyclin D2 (Fig. 6, A and B). These results agree with previous reports showing down-regulation of D-type cyclins during
mice, as opposed to 30% of BrdU+ cells in control mice. Thus, increased glucose metabolism via calcium entry can prevent the drop in cyclin D2 in β-cells during S-G2-M phases of the cell cycle (Fig. 6C).

To further study the basis for cyclin D2 down-regulation during β-cell replication, we took advantage of a novel transgenic mouse strain that we have recently generated. In these mice, replicating cells in the S-G2-M phases express GFP and can be sorted live by flow cytometry for molecular analysis (Klochendler, A., N. Weinberg-Corem, A. Eden, and Y. Dor, unpublished observations). Analysis of RNA extracted from sorted islet cells showed that cyclin D1 and cyclin D3 do not change during the cell cycle (data not shown). However, cyclin D2 mRNA is down-regulated during S-G2-M phases of the cell cycle (Fig. 6D). Surprisingly, we found that the mRNA of GCK was also down-regulated in replicating islet cells (Fig. 6D). These results suggest that cyclin D2 mRNA (and protein) levels are reduced in β-cells as they enter the S phase of the cell cycle. They also suggest the provocative idea that the basis for this phenomenon is a decrease in the level of GCK in replicating β-cells, impacting a signaling cascade as shown above for low extracellular glucose.

**Discussion**

We conclude that high levels of cyclin D2 in quiescent pancreatic β-cells are maintained by calcium entry downstream of GCK-mediated glycolysis. Furthermore, in replicating β-cells, cyclin D2 is down-regulated during S-G2-M phases of the cell cycle, via a similar mechanism sensitive to glucose metabolism. In both cases, regulation is exerted at the mRNA level.

Cyclin D2 has been shown to be expressed at high levels in pancreatic β-cells and to be important for β-cell replication, but how cyclin D2 expression is regulated has remained unclear. Although some reports have suggested that cyclin D2 is found in the cytoplasm and is localized to the nucleus upon replication (25, 27), others found that cyclin D2 is localized in the nuclei of most β-cells (21). Here, we have conclusively shown that cyclin D2 is constitutively localized in the nuclei of most β-cells.

Additionally, although several signaling pathways have been shown to impact the level of cyclin D2 in β-cells (22–24), the physiological mechanism accounting for the main-
decrease in cyclin D2 in replicating acinar cells, do not express cyclin D2. Image is taken from a 1-month-old mouse, injected with BrdU 2 h before killing. At least three animals with a minimum of 1500 BrdU -cells (−75 Brdu+ cells) were analyzed. ANOVA yielded *P* < 0.01. C, Enhanced glucose metabolism and intracellular calcium prevents the decline of cyclin D2 in replicating β-cells. One-month-old mice were injected with BrdU 8 h before killing. Seven hours before killing, mice were injected with a small molecule GKA and the calcium channel opener BayK8644. At least three animals with a minimum of 1500 β-cells (−75 Brdu+ cells) were analyzed. ANOVA yielded *P* < 0.01. D, Islet cells during S-G2-M phases of the cell cycle (GFP+) show reduced levels of cyclin D2 and GCK mRNA.

FIG. 6. Cyclin D2 is down-regulated in replicating β-cells via a glucose-sensitive mechanism. A, Costaining for cyclin D2 and BrdU reveals that replicating β-cells, as well as replicating acinar cells, do not express cyclin D2. Image is taken from a 1-month-old mouse, injected with BrdU 2 h before killing. B, Quantification of the fraction of cyclin D2+ β-cells in BrdU− β-cells in vivo and in BrdU+ β-cells in mice injected with BrdU 2 or 7 h before killing. At least three animals with a minimum of 1500 β-cells (−75 Brdu+ cells) were analyzed. ANOVA yielded *P* < 0.01. C, Enhanced glucose metabolism and intracellular calcium prevents the decrease in cyclin D2 in replicating β-cells. One-month-old mice were injected with BrdU 8 h before killing. Seven hours before killing, mice were injected with a small molecule GKA and the calcium channel opener BayK8644. At least three animals with a minimum of 1500 β-cells (−75 Brdu+ cells) were analyzed. ANOVA yielded *P* < 0.01. D, Islet cells during S-G2-M phases of the cell cycle (GFP+) show reduced levels of cyclin D2 and GCK mRNA.

We show that β-cell glucose metabolism is responsible for the basal level of cyclin D2 expression. Indeed, decreasing circulating glucose levels in vivo and reducing glucose levels in vitro caused a dramatic down-regulation in β-cell cyclin D2 levels. Moreover, we demonstrated that β-cell calcium channels are responsible for controlling the levels of cyclin D2, downstream of glycolysis. Calcium channel openers are able to rescue cyclin D2 down-regulation in low glucose conditions, and calcium channel blockers are able to lower the levels of β-cell cyclin D2 in high glucose. Our results also suggest that paracrine or autocrine insulin signaling downstream of calcium influx are not involved in cyclin D2 activation.

Previous studies have suggested that β-cell cyclin D2 may be principally regulated by a posttranslational mechanism (23, 25, 27). In contrast to these reports, we show that glucose controls β-cell cyclin D2 at the mRNA level, with minimal evidence for glucose regulation at the protein level.

Interestingly, although cyclin D2 is important for β-cell proliferation, its overexpression does not trigger β-cell replication (27). Although it has been suggested that glucose induces β-cell replication via increased cyclin D2 levels, our results suggest that cyclin D2 down-regulation may be important in the decline of β-cell proliferation in response to hypoglycemia and decreased intracellular calcium signaling. Further studies are necessary to examine whether overexpression of cyclin D2 can rescue the decline of β-cell proliferation in response to low glucose. Most importantly, it remains unknown what triggers the entry of quiescent β-cells into the cell division cycle. Our results argue that in contrast to what has been suggested, mitogen-induced induction of cyclin D2 is unlikely to be the key trigger for replication. Rather, cyclin D2 appears to have a permissive role; in other words, it is necessary but not sufficient for β-cell replication. This conclusion is consistent with the findings of He et al. (27), that high levels of cyclin D2 are sufficient to drive β-cell replication. We speculate that other components of the cell cycle machinery are responsible for the rare mitogen-induced switch from quiescence to replication in β-cells. Interesting candidates for this key process...
may include the down-regulation of cyclin kinase inhibitors or the up-regulation of CDK4/6.

The significance of cyclin D2 down-regulation during S-G2-M phases of the cell division cycle in β-cells remains unclear. Replicating cells in the testes and ovaries showed a similar dynamics, arguing that this is a general phenomenon (data not shown). At least in β-cells, it appears that this process is triggered by reduced rate of glycolysis during the cell cycle, potentially due to decreased expression of GCK mRNA. Further studies will be needed to characterize glucose metabolism during β-cell replication in vivo, its impact on cyclin D2 expression, and the function of cyclin D2 down-regulation during S-G2-M phases.

Although we demonstrate that glucose controls cyclin D2 mRNA levels in β-cells via calcium channels, additional studies will be needed to determine the responsible signaling pathway downstream of calcium. Notably, previous studies have shown that cyclin D2 in β-cells can be controlled by calcineurin/NFAT signaling (24) as well as STAT activity (26). Both of these pathways are likely activated by glycolysis via calcium in β-cells and thus could represent a molecular link between blood glucose levels and expression of β-cell cyclin D2.

Acknowledgments
We thank Jake Kushner and Anil Bhushan for providing sections of pancreata from cyclin D2−/− mice; Tatsuya Kin (Clinical Islet Isolation Laboratory, the University of Alberta) for the preparation of human islets; and Tomer Nir, Michael Brandeis, Oded Meyuhas, Ittai Ben-Porath, Robert Screaton, and Jens Nielsen for discussions and for sharing reagents.

Address all correspondence and requests for reprints to: Dr. Yuval Dor. Department of Developmental Biology and Cancer Research, The Institute for Medical Research Israel-Canada, Hadassah-Hebrew University Medical Center, Jerusalem 91120, Israel. E-mail: yuvald@ekmd.huji.ac.il.

This work was supported by National Institutes of Health β-Cell Biology Consortium, Juvenile Diabetes Research Foundation, Israel Science Foundation, Israel Cancer Research Fund, the European Union Seventh Framework Program Grant 241883, the Helmsley Foundation, and the Dutch friends of Hebrew University.

Disclosure Summary: The authors have nothing to disclose.

References


Members receive **Endocrine Daily Briefing**, an email digest of endocrinology-related news selected from thousands of sources.

www.endo-society.custombriefings.com