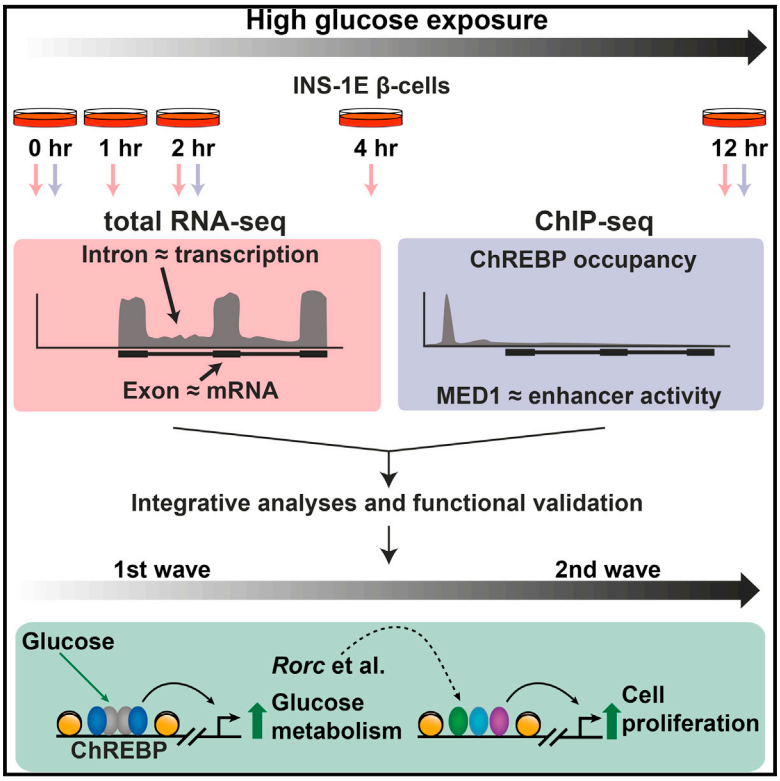
Article

Cell Reports

Integrative Genomics Outlines a Biphasic Glucose Response and a ChREBP-RORγ Axis Regulating Proliferation in β Cells

Graphical Abstract



Highlights

- Glucose reprograms the transcriptional network of INS-1E β cells in a biphasic manner
- ChREBP is a central regulator of the first wave of the glucose response
- Induction of cell-cycle genes requires ChREBP-induced transcription factors
- RORγ is required for full glucose-induced proliferation of INS-1E and primary β cells

Authors

Søren Fisker Schmidt, Jesper Grud Skat Madsen, Kari Østerli Frafjord, ..., Pierre Maechler, Louise Torp Dalgaard, Susanne Mandrup

Correspondence

s.mandrup@bmb.sdu.dk

In Brief

Schmidt et al. characterize the transcriptional reprogramming of the β cell enhancer and gene landscape by glucose and outline a ChREBP-initiated, biphasic response. Delayed induction of cell-cycle genes is mediated by secondary transcription factors including RORγ, which is required for full induction of β cell proliferation by glucose.

Accession Numbers

GSE81628

Schmidt et al., 2016, Cell Reports *16*, 2359–2372
August 30, 2016 © 2016 The Author(s).
http://dx.doi.org/10.1016/j.celrep.2016.07.063

CellPress

Cell Reports
Article

OPEN ACCESS
CellPress

Integrative Genomics Outlines a Biphasic Glucose Response and a ChREBP-RORγ Axis Regulating Proliferation in β Cells

Søren Fisker Schmidt,[1,6,7] Jesper Grud Skat Madsen,[1,2,6] Kari Østerli Frafjord,[1] Lars la Cour Poulsen,[1,8] Sofia Salö,[3] Michael Boergesen,[1,9] Anne Loft,[1,7] Bjørk Ditlev Larsen,[1] Maria Stahl Madsen,[1] Jens Juul Holst,[2,4] Pierre Maechler,[5] Louise Torp Dalgaard,[3] and Susanne Mandrup[1,10,*]

[1]Department of Biochemistry and Molecular Biology, University of Southern Denmark, 5230 Odense M, Denmark
[2]NNF Center of Basic Metabolic Research, University of Copenhagen, 2200 Copenhagen N, Denmark
[3]Department of Science and Environment, Roskilde University, 4000 Roskilde, Denmark
[4]Department of Biomedical Sciences, University of Copenhagen, 2200 Copenhagen N, Denmark
[5]Department of Cell Physiology and Metabolism, University of Geneva, 1211 Geneva, Switzerland
[6]Co-first author
[7]Present address: Institute of Diabetes and Cancer (IDC), Helmholtz Center Munich, German Research Center for Environmental Health, 85764 Neuherberg, Germany
[8]Present address: Department of Pathology, Oslo University Hospital, Rikshospitalet, 0027 Oslo, Norway
[9]Present address: PentaBase Aps, 5000 Odense C, Denmark
[10]Lead Contact
*Correspondence: s.mandrup@bmb.sdu.dk
http://dx.doi.org/10.1016/j.celrep.2016.07.063

SUMMARY

Glucose is an important inducer of insulin secretion, but it also stimulates long-term adaptive changes in gene expression that can either promote or antagonize the proliferative potential and function of β cells. Here, we have generated time-resolved profiles of enhancer and transcriptional activity in response to glucose in the INS-1E pancreatic β cell line. Our data outline a biphasic response with a first transcriptional wave during which metabolic genes are activated, and a second wave where cell-cycle genes are activated and β cell identity genes are repressed. The glucose-sensing transcription factor ChREBP directly activates first wave enhancers, whereas repression and activation of second wave enhancers are indirect. By integrating motif enrichment within late-regulated enhancers with expression profiles of the associated transcription factors, we have identified multiple putative regulators of the second wave. These include RORγ, the activity of which is important for glucose-induced proliferation of both INS-1E and primary rat β cells.

INTRODUCTION

Pancreatic β cells play a key role in the regulation of glucose uptake and metabolism. They respond to a rise in blood glucose by increasing the secretion of insulin, which in turn increases the storage and utilization of glucose in peripheral tissues. Type 2 diabetes (T2D) is caused by an inability of the β cells to secrete sufficient amounts of insulin to meet the demands for nutrient use and storage. Insulin resistance in liver and peripheral tissues contributes significantly to this imbalance, but whether insulin resistant subjects develop hyperglycemia appears to depend on their ability to expand the β cell mass or capacity in response to the increased metabolic load. Conversely, β cells of type 2 diabetics respond poorly to a glucose challenge; however, the etiology of β cell dysfunction in T2D remains controversial. Traditionally, the deficit in β cell capacity has been ascribed to increased apoptosis, but recent studies suggest that β cell dedifferentiation plays a dominant role in β cell failure in T2D (Talchai et al., 2012). Interestingly, during the progression to insulin resistance and T2D, it appears that glucose itself acts both to promote expansion of β cell mass to meet higher demands of insulin (Levitt et al., 2011; Lingohr et al., 2002) and as a stressor that can lead to dysfunction, so-called glucotoxicity (Brun et al., 2015; Prentki and Nolan, 2006). However, so far the changes in β cell gene expression underlying these adaptive and pathogenic effects are far from understood, and genome-wide insight into transcriptional reprogramming of the β cell genome by glucose is missing.

Several recent studies have implicated the transcription factor carbohydrate response element binding protein (ChREBP) in glucose-induced regulation of β cell gene expression and function (Boergesen et al., 2011; da Silva Xavier et al., 2006; Metukuri et al., 2012; Poungvarin et al., 2012). ChREBP is a basic helix-loop-helix leucine zipper (bHLH-LZ) transcription factor that exerts its actions by binding to DNA on carbohydrate response elements (ChoREs) together with another bHLH-LZ protein called Max-like protein X (Mlx) (Ma et al., 2005). ChREBP senses glucose by multiple different mechanisms, which vary between cell types, but most signaling converge onto a glucose sensing module in the N terminus, the deletion of which renders ChREBP

This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

constitutively active (Boergesen et al., 2011; Li et al., 2006). ChREBP has been shown to promote both glucose-induced β cell proliferation (Metukuri et al., 2012) and dysfunction (Poungvarin et al., 2012), thereby reflecting the dual effects of glucose on β cell function. However, while a role for ChREBP at these and additional functional levels has been demonstrated, only a few ChREBP target genes are known in β cells, and the mechanistic understanding of these pleiotropic effects of ChREBP is incomplete.

Here, we have generated time-resolved profiles of the transcriptional response to glucose in INS-1E pancreatic β cells and used gain- and loss-of-function studies and chromatin immunoprecipitation sequencing (ChIP-seq) to dissect the role of ChREBP herein. Our data outline a ChREBP-initiated, biphasic response with acute (1–2 hr) induction of genes involved in glucose and lipid metabolism and delayed (4–12 hr) induction of cell-cycle genes along with repression of β cell identity genes. By integrative bioinformatics analyses, we have identified multiple drivers of the cell-cycle gene program, including RAR-related orphan receptor (ROR) γ, which we demonstrate is a direct ChREBP target gene important for induction of β cell proliferation by glucose.

RESULTS

Transcriptional Reprogramming of INS-1E β Cells following High Glucose Exposure

To map the transcriptional response to high glucose in β cells, we preincubated INS-1E cells at 5 mM glucose for 24 hr and performed total RNA-seq following 0, 1, 2, 4, and 12 hr exposure to 25 mM glucose. Exposure to high glucose for 12 hr led to a pronounced reprogramming of the INS-1E cell transcriptome with 2,042 and 1,700 genes significantly induced and repressed, respectively (FDR $\leq$ 0.05) (Figure 1A). The INS-1E transcriptome is similar to that of rat pancreatic islets (Figures S1A–S1C) (Bensellam et al., 2009) compared to other tissues in the rat body map (Yu et al., 2014) and the glucose-induced changes in gene expression are comparable between INS-1E cells and rat pancreatic islets (Figures S1D–S1F) (Bensellam et al., 2009). Interestingly, genes induced by glucose in INS-1E cells are also enriched among genes that are expressed to higher levels in islets from type 2 diabetics compared with islets from non-diabetic controls, whereas genes repressed by glucose are enriched among genes that are expressed to lower levels in diabetic compared with non-diabetic islets (Figures S1G and S1H).

Pathway analysis revealed that genes induced by glucose in INS-1E cells are enriched in glucose metabolism and cell-cycle pathways, whereas genes repressed by glucose among others are associated with insulin secretion and maturity-onset diabetes of the young (MODY) (Figure 1B). To investigate the changes in transcriptional activity underlying the glucose-induced reprogramming of the β cell transcriptome, we used our recently developed computational method, iRNA-seq (Madsen et al., 2015). iRNA-seq identifies reads that map uniquely to introns of each gene and estimates changes in transcription levels between conditions based on this information. These changes correlate well with those determined by other methods for assessment of transcriptional activity (Madsen et al., 2015; Schmidt et al., 2015). In line with these recent studies, analysis of intron reads allowed for much better detection of acute gene regulation by glucose compared with exon reads (Figures S1I–S1K). We identified in total 5,302 genes that were significantly regulated at the primary or mature transcript levels between 0 hr and one or more of the other time points; and using fuzzy cluster analysis, these genes were grouped into four clusters based on temporal changes in primary transcript (Figure 1C). The four clusters display enrichment of distinct metabolic pathways with genes acutely induced by high glucose (cluster 1) being enriched in glucose and steroid metabolism pathways, whereas cell-cycle genes are enriched in the late-induced cluster (cluster 2), and genes involved in insulin secretion and MODY are enriched in the late-repressed cluster (cluster 4) (Figure 1C; Table S1). Consistently, gene cluster 4 displays a higher overlap with genes expressed specifically in pancreatic islets (Table S2) (http://www.betacell.org/) compared with constitutive genes and the three other gene clusters (Figure 1D). Interestingly, acutely induced genes are more likely to also be induced by glucose in hepatocytes (Ma et al., 2006) than the late-induced genes that appear to be induced by glucose in a β cell specific manner (Figure 1E). Collectively, our temporal genome-wide analysis of the transcriptional response to glucose in pancreatic β cells reveals a biphasic transcriptional response leading to acute induction of genes involved in glucose and lipid metabolism, delayed activation of proliferative genes, and delayed repression of genes involved in β cell function.

ChREBP Is a Central Mediator of Glucose-Induced Transcriptional Reprogramming in β Cells

To investigate the role of ChREBP in the global transcriptional reprogramming of β cells by glucose, we transduced INS-1E cells with an adenovirus expressing an N-terminally truncated constitutive active ChREBP (Ad-CA-ChREBP) (Boergesen et al., 2011) or GFP (Ad-GFP) as a control. Notably, analysis of total RNA-seq data from Ad-CA-ChREBP and Ad-GFP transduced INS-1E revealed that CA-ChREBP is capable of regulating the majority of the glucose-regulated genes in β cells (Figures 2A and 2B) including genes involved in cell proliferation and MODY/insulin secretion (Figures 2C and 2D). Next, we transfected INS-1E cells with small interfering (si)RNA targeting ChREBP (siChREBP) or a negative control (siControl), resulting in robust knock down of ChREBP, and decreased glucose-induction of well-known ChREBP target genes such as *Pklr* and *Gpd1* (Figures S2A and S2B). mRNA-seq profiling of the response to 12 hr of high glucose in siChREBP- and siControl-cells revealed that ChREBP depletion efficiently blunts the regulation of gene programs by glucose (Figures 2E, 2F, and S2C). Collectively, these data place ChREBP as a key regulator of glucose-induced transcriptional reprogramming of β cells.

Glucose Induces Reprogramming of Enhancer Activity and Architecture

To investigate the transcriptional mechanisms underlying reprogramming of the β cell transcriptome by glucose, we first determined the effects of 2 and 12 hr glucose exposure on enhancer activity in INS-1E cells. Using mediator complex

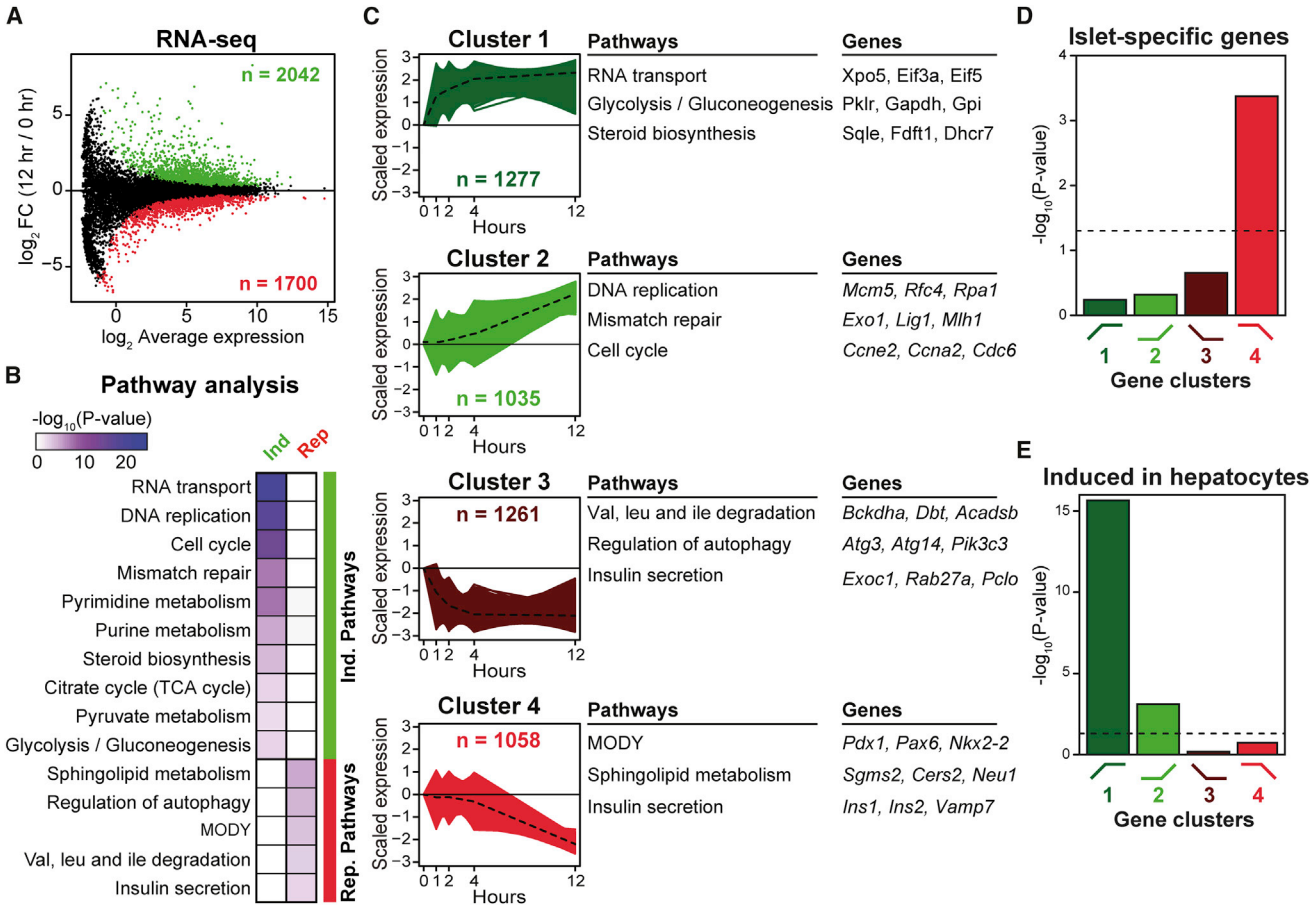


Figure 1. High Glucose Induces Biphasic Transcriptional Changes in INS-1E β Cells

Following 24 hr preincubation in 5 mM glucose medium, INS-1E cells were switched to fresh 5 mM medium, and 25 mM glucose was added for 1, 2, 4, and 12 hr in a reverse time course before simultaneous harvest of all samples for total RNA-seq. Changes in mRNA expression and transcriptional activity were determined using the iRNA-seq pipeline.

(A) MA-plot plot showing mean expression level versus $\log_2$ fold change (induced by 12 hr high glucose exposure) in tag count within exons of Ensembl gene bodies. The green and red dots represent genes that were determined to be induced and repressed, respectively (FDR $\leq$ 0.05).

(B) Heatmap representing -$\log_{10}$(p value) for the top enriched pathways for genes induced and repressed by high glucose (12 hr versus 0 hr).

(C) Fuzzy cluster analysis of the temporal changes in transcriptional activity (intron reads) of all genes with dynamic expression pattern (i.e., with difference [FDR $\leq$ 0.05] between 0 hr and any time point at either nascent or mature transcript level). The top three enriched pathways and gene examples are listed for each cluster.

(D and E) Bar diagrams illustrating the significance in a one-tailed chi-square test of the overlap between the four gene clusters defined in (C) and lists of islet-specific genes (D) and a list of genes induced by glucose in primary hepatocytes (E) compared with the overlap between the different lists and constitutive genes. (E: cluster 1 versus cluster 2: p = 0.003).

subunit 1 (MED1) ChIP-seq as a surrogate for enhancer activity (Heintzman et al., 2009), we identified in total 20,632 putative enhancers with a significant change in activity between the 0 hr and the 2 and/or 12 hr time points (Figures S3A and S3B). Using fuzzy cluster analysis, we grouped these regions into six clusters with distinct glucose-induced temporal changes in enhancer activity (Figure 3A). For all enhancer clusters, we found that glucose-induced changes in MED1 occupancy are associated with corresponding changes in enhancer RNA (eRNA) transcription (Kim et al., 2010) and in chromatin accessibility, as assessed by DNase I hypersensitive sites (DNase-) seq (Crawford et al., 2006) (Figures 3B and S3C–S3F). Importantly, MED1 clusters are enriched in the vicinity of genes that are regulated in a similar temporal manner (Figure 3C), as illustrated for the acutely activated *Pklr* gene (Figure 3D).

ChREBP Is Acutely and Fully Activated by Glucose and Primarily Associates with Acutely Activated Enhancers

To further investigate the mechanisms by which ChREBP mediates glucose-induced enhancer and gene reprogramming in β cells, we performed ChREBP ChIP-seq in INS-1E cells exposed to high glucose for 0, 2, and 12 hr and identified in total 74,967 ChREBP binding sites (Figure 3E). De novo motif discovery performed on the top 5,000 ChREBP sites identified a degenerate CACGTGn_5CACGTG ChoRE resembling those previously discovered by others (Figure 3F) (Jeong et al., 2011; Ma et al.,

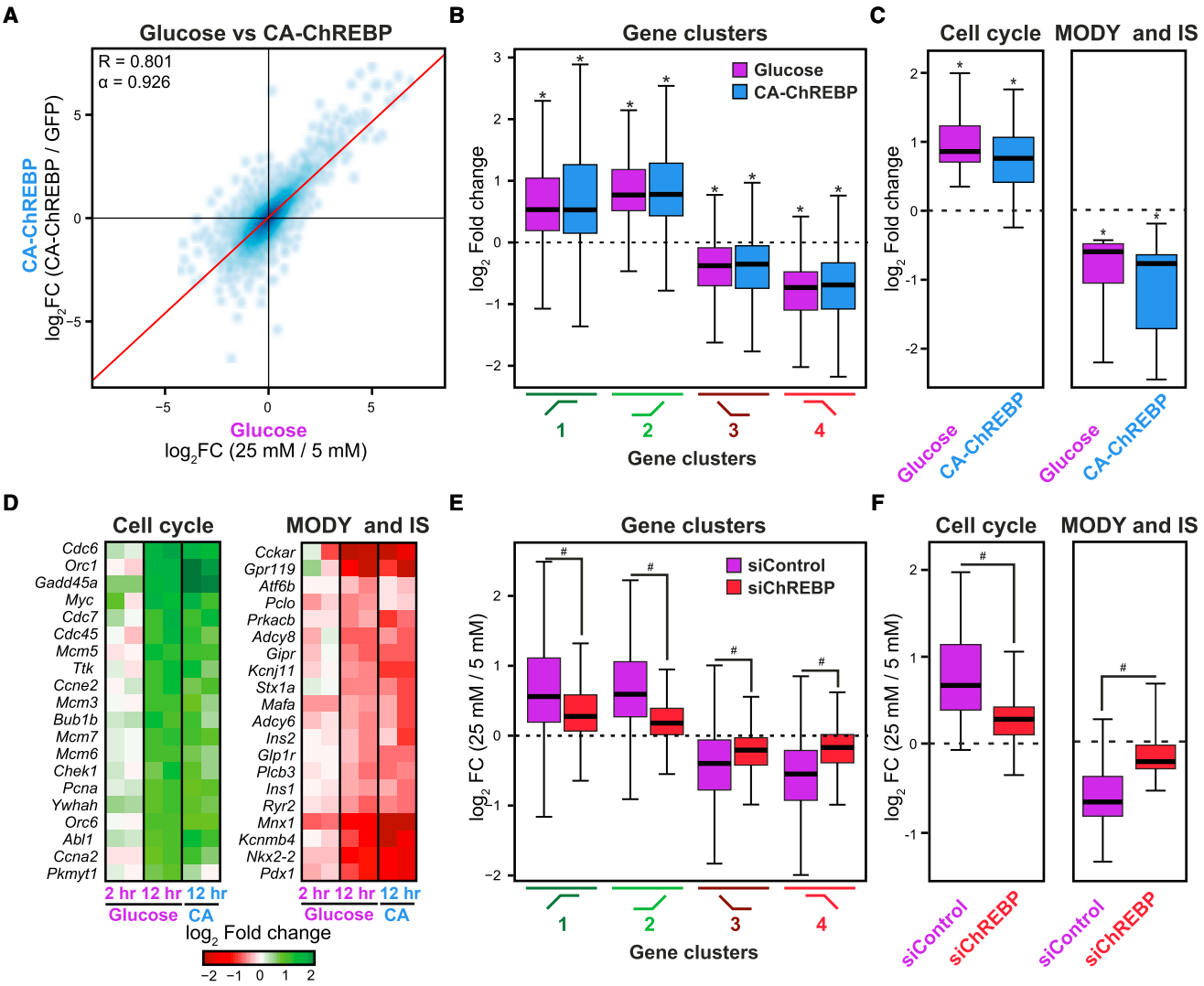


Figure 2. ChREBP Is a Central Mediator of Glucose-Induced Transcriptional Reprogramming in β Cells

(A–D) INS-1E cells were preincubated with 5 mM glucose for 24 hr and subsequently transduced with equal titers of adenovirus expressing constitutive active ChREBP (CA-ChREBP) or GFP as control. After 2 hr, medium was replaced with fresh 5 mM medium, and INS-1E cells were harvested for total RNA-seq 12 hr later.

(A) Density plot illustrating the correlation between expression fold-change (mRNA) induced by 25 mM glucose (Figure 1) and CA-ChREBP. The red line shows the linear regression (Pearson).

(B and C) Boxplots illustrating expression fold change (mRNA) induced by 25 mM glucose (purple) and CA-ChREBP (blue) for genes in each of the four gene clusters defined in Figure 1C (B) and for glucose-regulated genes in the cell-cycle pathways (C, left) and MODY/insulin secretion (IS) pathways (C, right) (*$p < 2.2 \times 10^{-16}$, Wilcoxon rank-sum test relative to 0).

(D) Heatmaps illustrating expression fold change (mRNA) induced by 2 hr glucose, 12 hr glucose, and CA-ChREBP for the top 20 glucose-regulated genes in the cell-cycle pathways (D, left) and MODY/insulin secretion (IS) pathways (D, right).

(E and F) INS-1E cells were transfected with siRNAs targeting ChREBP (siChREBP, red) or a negative control (siControl, purple) and 2 days later preincubated with 5 mM glucose for 24 hr and subsequently treated with 5 mM or 25 mM glucose for 12 hr before harvest of RNA for mRNA-seq. The boxplots illustrate expression fold change (mRNA) for genes induced by high glucose in siControl and siChREBP transfected cells for each of the four gene clusters defined in Figure 1C (E) and for glucose-regulated genes in the cell cycle pathways (F, left) and MODY/insulin secretion (IS) pathways (F, right) (#$p < 2.2 \times 10^{-16}$, Wilcoxon rank-sum test).

2006; Poungvarin et al., 2015). A series of electrophoretic mobility shift and reporter assay studies by the Towle laboratory have indicated that whereas ChREBP is capable of binding to perfect single E-boxes as a heterodimer with Mlx, transcriptional activation by glucose requires binding as a heterotetramer to the full ChoRE (Ma et al., 2005, 2007; Stoeckman et al., 2004). The combination of ChREBP and MED1 ChIP-seq data allowed us to reinvestigate this in a natural chromatin context by computational mutation analysis. Specifically, we identified the best possible match to a perfect ChoRE in each ChREBP site, divided ChREBP sites into groups based on the number of mismatches to each E-Box (Figure S3G), and calculated average ChREBP

binding and glucose-induced change in MED1 binding for each of these groups. These analyses confirmed in a natural chromatin context and at the genome-wide level that given a perfect CACGTG E-box, ChREBP binding is largely independent of the number of mismatches in the second E-Box, whereas glucose-induced enhancer activation is highly sensitive to these mismatches (Figures 3G and S3H). Notably, our analyses did not support the previously reported role of ChREBP as a transcriptional repressor when binding to a single E-box (Cairo et al., 2001), since none of the groups displayed an overall loss of MED1 following glucose exposure.

Importantly, the ChIP-seq profiles also revealed that ChREBP binding is fully activated already following 2 hr of glucose exposure (Figures 3E and 3H), indicating that ChREBP is primarily involved in the first phase response to glucose. In line with this, ChREBP binding is primarily enriched within early-activated enhancers (Figures 3I and S3I) and in the vicinity of genes acutely induced by glucose (Figures 3J and S3J). Consistent with the results in Figure 3C, these analyses do not support a major global role of ChREBP as a transcriptional repressor, as ChREBP binding is not enriched at any of the glucose-repressed enhancer groups or near glucose-repressed genes (Figures 3I, 3J, S3I, and S3J). These results place ChREBP as a master regulator of primarily the first phase activation of genes in response to glucose and indicate that the late induction of, e.g., cell-cycle genes involves ChREBP-mediated induction of secondary transcription factors.

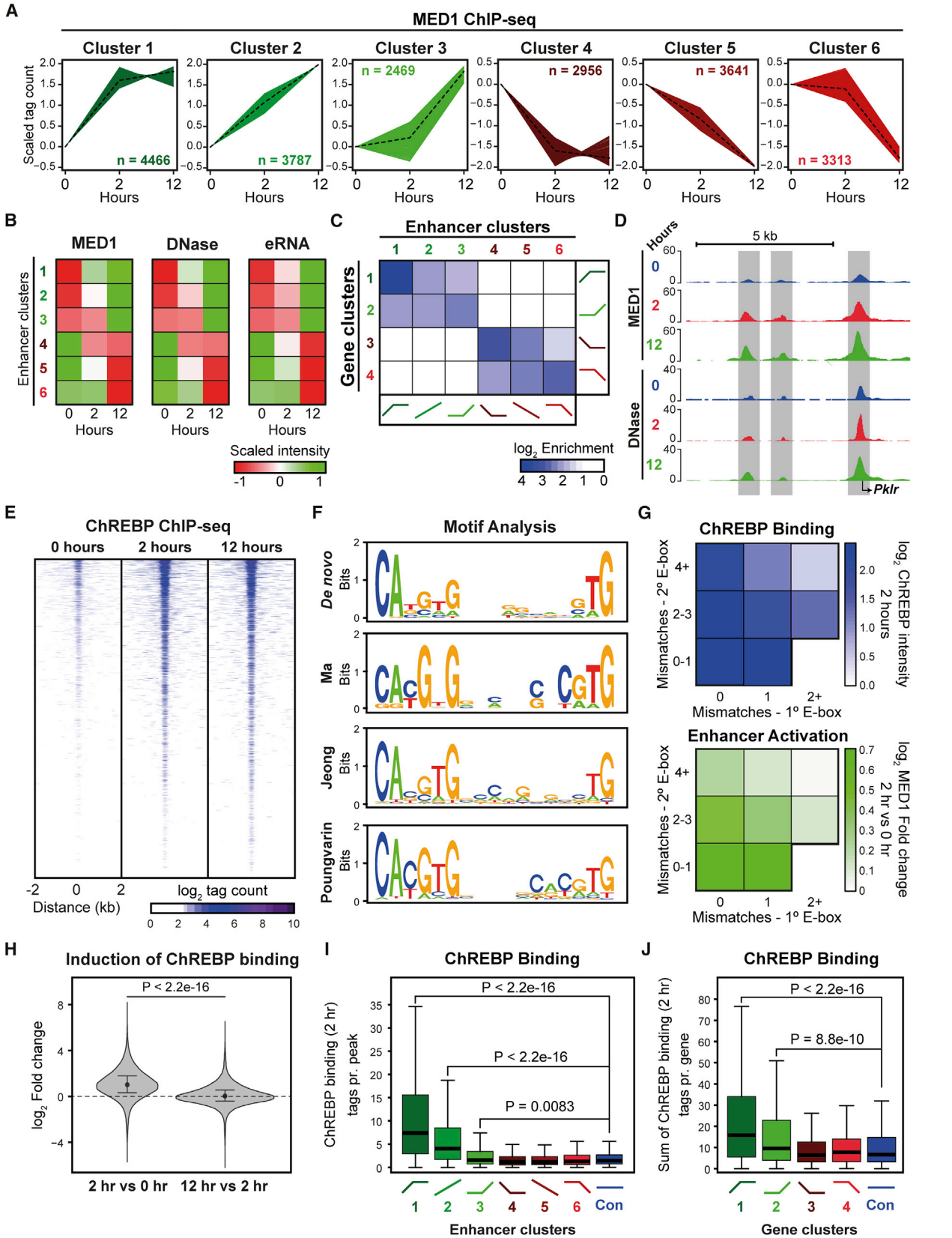
Integrated Motif and Expression Analysis Identifies Potential Regulators of β Cell Proliferation

To identify transcription factors involved in the second phase β cell response to glucose, we analyzed the DNA sequences underlying late regulated enhancers. General issues with such analyses are: (1) the bias imposed by the limited number of transcription factor motifs available in the database associated with the motif tool of choice; (2) the redundancy between transcription factors in binding motif; (3) choosing an appropriate motif threshold; and (4) identifying the biologically relevant hits from a rather large number of enriched motifs. To overcome these limitations, we curated and annotated several publically available motif databases and used a variable threshold approach to identify enriched motifs, which were subsequently systematically filtered based on the absolute and glucose-induced change in expression of the matching transcription factors (see Supplemental Information). Using this approach, we identified 234 motifs enriched within late repressed enhancers (cluster 6) and 52 motifs enriched in late activated enhancers (cluster 3) compared with both constitutive enhancers and randomly shuffled control regions (Figure 4A). A matching transcription factor was expressed (pr. kb $\geq$ 1) at any given time point for 47 and 28 of the motifs enriched within late repressed and activated enhancers, respectively. Reassuringly, the expression of transcription factors with motifs that are enriched within late repressed enhancers is generally repressed by glucose, whereas the expression of transcription factors with motifs that display enrichment in late activated enhancers is predominantly induced by glucose (Figure 4B) (Table S3). Notably, we identified 19 potential transcriptional activators of the late repressed enhancers, whose expression is also significantly downregulated by glucose and several of these are well known regulators of β cell identity and function (Aguayo-Mazzucato et al., 2013; Ait-Lounis et al., 2010; Barbagallo et al., 2014; Doyle and Sussel, 2007; Harrison et al., 1999; Heit et al., 2006; Holland et al., 2005; Jackerott et al., 2006; Kitamura, 2013; Moore et al., 2011; Ohta et al., 2011; Zitzer et al., 2006) (Figures 4B and 4C). In the same manner, we identified 13 potential activators of the late-induced enhancers, of which we chose five top candidates for further investigation based on their fold induction by glucose and their temporal profile of transcriptional activation (cluster 1) (Figure 4B). Interestingly, knock down of four out of five of these factors significantly reduced the induction of cell-cycle genes by glucose as determined by mRNA-seq (Figure 4D). This further supports a role of these glucose-induced transcription factors in mediating ChREBP-induced β cell proliferation. Notably, knock down of one of the four factors had limited if any effect on expression of the other factors (Figures S4A–S4D), indicating that these factors play relatively independent roles in the cell-cycle gene program. The fact that siBHLHE40 does not affect cell-cycle gene expression may be due to the poor knockdown efficiency of this siRNA pool (Figure S4E).

RORγ Is a Direct ChREBP Target Involved in Glucose-Induced β Cell Proliferation

Besides MYCN, which is a well-known regulator of cell proliferation (Bell et al., 2010), depletion of the nuclear receptor RORγ displayed the largest impact on glucose induction of cell-cycle genes. RORγ is a diurnal regulator with well-defined functions in liver and adipose tissues that appear to promote insulin resistance in mice fed an obesogenic diet (Meissburger et al., 2011; Takeda et al., 2014); however, little is known about the function of RORγ in β cells. Interestingly, we observed a prominent glucose-activated, ChREBP-bound enhancer 1 kb upstream of the promoter of the *Rorc* gene (Figure 5A) and this enhancer is also conserved in human pancreatic islets (Encode, 2012) and in isolated human primary β cells (Ackermann et al., 2016) (Figure 5B). The *Rorc* −1 kb enhancer contains several putative ChoREs that are highly conserved in the mouse and human genomes (Figures 5C, 5D, S5A, and S5B), suggesting that activation of *Rorc* expression by ChREBP involves cooperative binding to multiple ChoREs within this enhancer. Consistent with *Rorc* being a direct target gene of ChREBP, ChREBP depletion significantly blunts glucose induction of nascent *Rorc* transcript after both 2 hr and 12 hr (Figure 5E). The acute induction of nascent *Rorc* transcript levels by glucose is followed by accumulation of the mature mRNA of *Rorc* and in a further delayed manner of RORγ protein levels (Figures 5F and S5C), consistent with a role of RORγ in late gene regulation by glucose. The induction of *Rorc* expression by glucose is also observed in human pancreatic islets cultured ex vivo (Figures 5G and S5D).

Interestingly, further analyses of the mRNA-seq data obtained from siRORγ- and siControl-transfected INS-1E cells suggests a selective role of RORγ in activation of the late glucose-induced gene program including cell-cycle genes (Figures 6A and 6B). Transfection with siRORγ reduced RORγ protein levels by 80% (Figure S5C) and did not affect mRNA levels of *Rora* or *Rorb* (Figure S6). Importantly, the glucose-induced proliferation



(legend on next page)

rate of INS-1E β cells, assessed by bromodeoxyuridine (BrdU) incorporation, is significantly decreased in siRORγ-transfected cells (Figure 6C), and to test the relevance of this finding in primary β cells, we cultured isolated rat pancreatic islets for 48 hr in the presence of 5 or 20 mM glucose in combination with the RORγ antagonist SR2211 or DMSO. Indeed, SR2211 treatment significantly reduced glucose-induced β cell proliferation in rat islets as assessed by 5-ethynyl-2′-deoxyuridine (EdU) incorporation (Figures 6D and 6E). Taken together, these data support an important role for ChREBP-mediated induction of RORγ expression in the induction of β cell proliferation by glucose.

DISCUSSION

Glucose has profound long-term effects on β cell function that can lead to both increased proliferative potential and compromised β cell function. These adaptive changes have been reported to involve several different transcription factors including ChREBP; however, the molecular mechanisms underlying the pleiotropic effects of glucose and ChREBP are not well understood. Here, we profile changes in enhancer and transcriptional activity in response to glucose in pancreatic β cells, and we use these data to define a central role for ChREBP as initiator of the response and to identify transcription factors (TFs) acting downstream of ChRBEP. In particular, we identify RORγ as a ChREBP-induced regulator of β cell proliferation.

Similar to previous studies on the role of ChREBP in regulation of β cell proliferation and function (Metukuri et al., 2012; Poungvarin et al., 2012), we treated INS-1E cells with 25 mM glucose for up to 12 hr following preincubation at 5 mM glucose. This setup allows for detailed temporal analyses to dissect the transcriptional networks involved in establishing and/or acting in a situation of chronically elevated glucose levels. While the setup is not directly comparable to the more modest increase in blood glucose levels observed in diabetics that occurs progressively over much longer periods, the genes regulated by glucose in INS-1E cells tend to be also differentially expressed in a similar manner in islets from type 2 diabetic subjects relative to islets from healthy control subjects. This indicates that the glucose-treated INS-1E cells recapitulate at least some features of diabetic islets.

Our time-resolved profiles of changes in transcription and enhancer activity show that the response to glucose is biphasic. Genes involved in glucose and lipid metabolism are acutely activated in a first wave, which is followed by a second wave during which cell-cycle genes are activated and β cell identity genes are repressed. We demonstrate by gain- and loss-of-function experiments that ChREBP is involved in both gene activation and repression of genes in both waves, which is in line with recent studies that have implicated ChREBP in both the proliferative (Metukuri et al., 2012) and toxic (Poungvarin et al., 2012) effects of glucose on pancreatic β cells. However, our genome-wide analyses show that ChREBP binding is primarily associated with enhancers that are activated in the first wave, indicating that activation of the second wave enhancers as well as repression by ChREBP is mostly indirect. ChREBP has previously been reported to have repressor function (Boergesen et al., 2011; Cairo et al., 2001); however, the fact that ChREBP is not enriched at repressed enhancers in glucose-stimulated INS-1E cells does not support a major global role of ChREBP as a transcriptional repressor in the classical sense in this system. We consider it likely that acute repression occurs by cofactor redistribution as demonstrated for nuclear receptors and NFκB (Schmidt et al., 2016), but further experiments are needed to rule out contributions from alternative mechanisms such as deactivation of other TFs by high glucose.

Given the biphasic repression of gene expression, it is likely that decreased expression/activity of transcriptional activators acutely repressed by glucose over time leads to second wave repression. The strength in profiling enhancer activity is that we can predict TFs involved in the various phases of the response by bioinformatic analysis of motif enrichment within specific

Figure 3. MED1 and ChREBP ChIP-Seq in Glucose Stimulated INS-1E β Cells

Following 24 hr preincubation in 5 mM glucose medium, INS-1E cells were switched to fresh 5 mM medium and 25 mM glucose was added for 0, 2, and 12 hr in a reverse time course before harvest of all samples for ChIP-seq or DNase-seq at the same time.

(A) Fuzzy cluster analysis of the temporal changes in enhancer activity, as assessed by MED1 read counts, of all dynamic enhancers (i.e., enhancers with difference [FDR ≤ 0.05] between 5 mM glucose and 2 or 12 hr of stimulation with 25 mM glucose).

(B) Heatmaps illustrating average MED1 signal, DNase I accessibility, and eRNA levels for the six different MED1 clusters of regulated enhancer in INS-1E cells treated with high glucose for 0, 2, or 12 hr.

(C) Heatmap illustrating location of enhancer clusters defined in (A) relative to the gene clusters defined in Figure 1C. The enrichment of the six different enhancer clusters within 50 kb of the transcription start site (TSS) of genes in the four gene clusters is plotted relative to the number of enhancers found near constitutive genes.

(D) UCSC Genome Browser screenshots of MED1- and DNase I signal at the gene locus of a typical cluster 1 gene *Pklr*.

(E) Heatmap showing ChREBP binding intensity in a 4 kb window around the center of all identified ChREBP sites in INS-1E cells treated with high glucose (25 mM) for 0, 2, and 12 hr.

(F) Motif identified by de novo motif analysis of the top 5,000 identified ChREBP binding sites (top). The ChREBP binding motifs identified in three different studies: Ma et al. (2006), Jeong et al. (2011), and Poungvarin et al. (2015) (below) are shown.

(G) Heatmaps representing motif dependency for ChREBP binding and enhancer activation by glucose. All identified ChREBP binding sites were grouped based on the number of mismatches of the best possible ChoRE half site in each site compared with a perfect ChoRE. See also Figure S3G. The average ChREBP binding intensity (G, top) and $\log_2$ fold change in MED1 occupancy in response to 2 hr exposure to high (25 mM) glucose (G, bottom) were determined for each group of binding sites.

(H) Violin plot representing fold change in ChREBP binding in response to 25 mM glucose exposure for 2 versus 0 hr (H, left) and 12 versus 2 hr (H, right) of.

(I) Boxplots illustrating ChREBP occupancy (2 hr) at glucose-regulated enhancer clusters defined in (A).

(J) Boxplots representing summarized ChREBP occupancies (2 hr) at all ChREBP binding sites within 50 kb of the TSS of glucose-regulated gene clusters defined in Figure 1C. The indicated p values were calculated using the Wilcoxon rank-sum test.

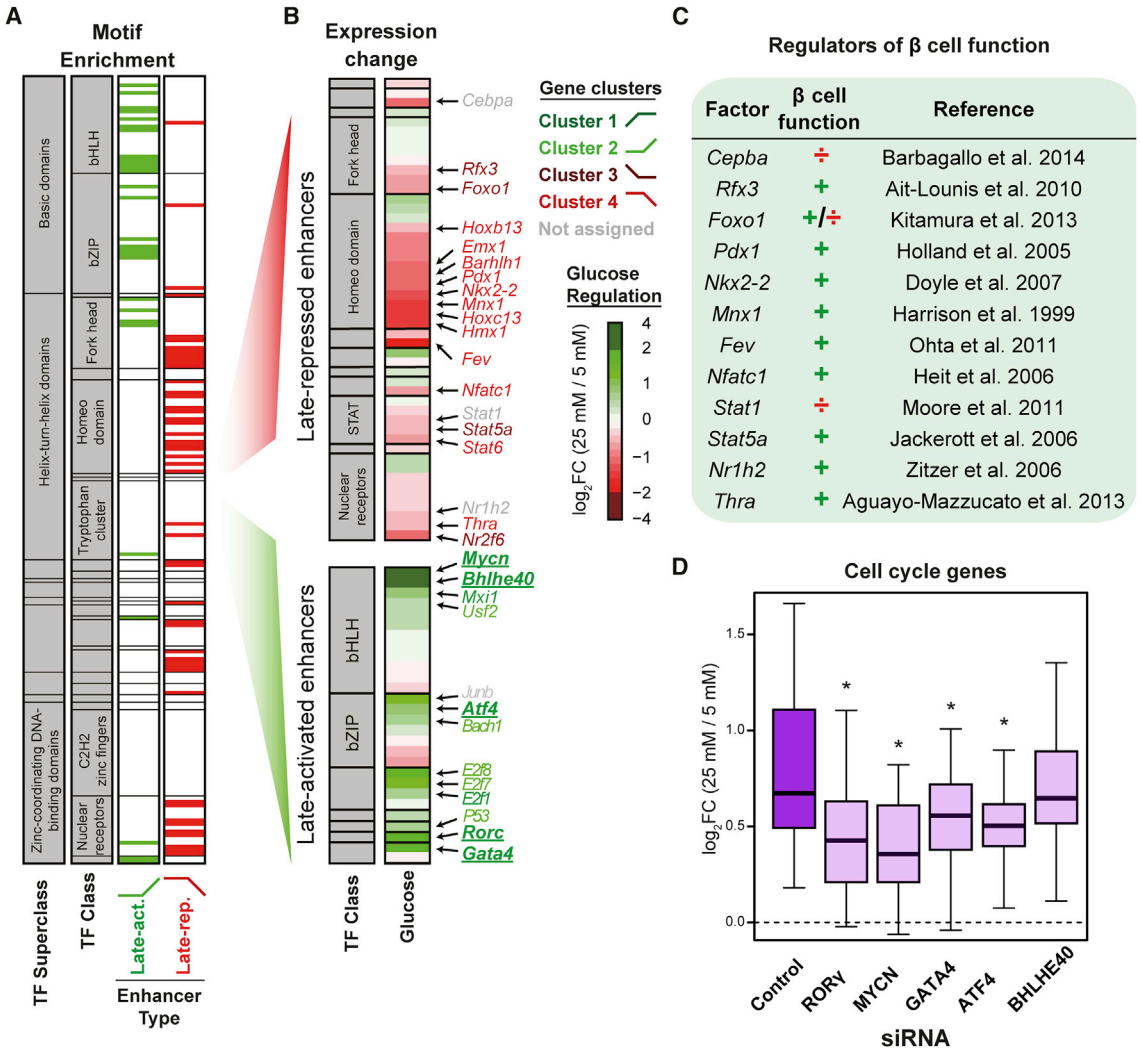


Figure 4. Identification of Potential Second Wave Regulators by Integrated Motif and Expression Analysis

(A) Heatmap representing motifs enriched within late repressed (cluster 6, red) or activated (cluster 3, green) enhancers as defined in Figure 3A.

(B) Heatmap illustrating fold change in expression (mRNA, 12 versus 0 hr) for TFs with enriched motifs in late repressed (B, top) or late activated (B, bottom) enhancers. TFs whose expression is significantly regulated by glucose in the same direction as the activity of the enhancer cluster in which their motif is enriched are listed. The listed TFs are color coded according to the temporal profile of their primary transcript levels as defined in Figure 1C.

(C) Table listing potential activators of late repressed enhancers with known regulatory roles of β cell gene expression, identity, and function.

(D) INS-1E cells were transfected with a negative control siRNA (siControl, dark purple) and siRNAs targeting five potential activators of the second wave enhancers activated by glucose identified in (A) and (B) (light purple). 2 days later, cells were preincubated with 5 mM glucose for 24 hr and subsequently treated with 5 mM or 25 mM glucose for 12 hr before harvest of RNA for mRNA-seq. The boxplot illustrates expression fold change (mRNA) induced by glucose in control and knockdown cells for glucose-activated genes in the cell-cycle pathway (*$p < 2.2 \times 10^{-16}$, Wilcoxon rank-sum test relative to siControl).

enhancer clusters. In this work, we have used an approach that integrates motif enrichment with expression profiles of the corresponding TFs, thereby improving the prediction of TFs involved in changing enhancer activity. The approach relies on the fact that most mammalian TFs act to promote transcription at their binding sites (Hurst et al., 2014) and identifies factors whose mRNA expression is regulated congruently with the activity of the temporal cluster of enhancers in which the corresponding motif is enriched. The approach does not take changes in TF activity by posttranslational modifications into consideration, but focuses on mRNA expression of the TF as an estimate of activity. Thus, the approach is particularly useful for identifying regulators of the second wave transcriptional response, because these are likely to be transcriptionally modulated by the first wave TFs. By this approach, we could reduce a list of 234 TF with motifs enriched within late repressed enhancers to a set of 18 potential transcriptional regulators of the late repressed enhancers whose expression is also significantly downregulated by glucose.

Interestingly, and as a validation of our approach, the potential regulators of late repressed enhancers include well-known regulators of β cell identity like PDX1 (Holland et al., 2005) and NKX2-2 (Doyle and Sussel, 2007). Among the acutely repressed

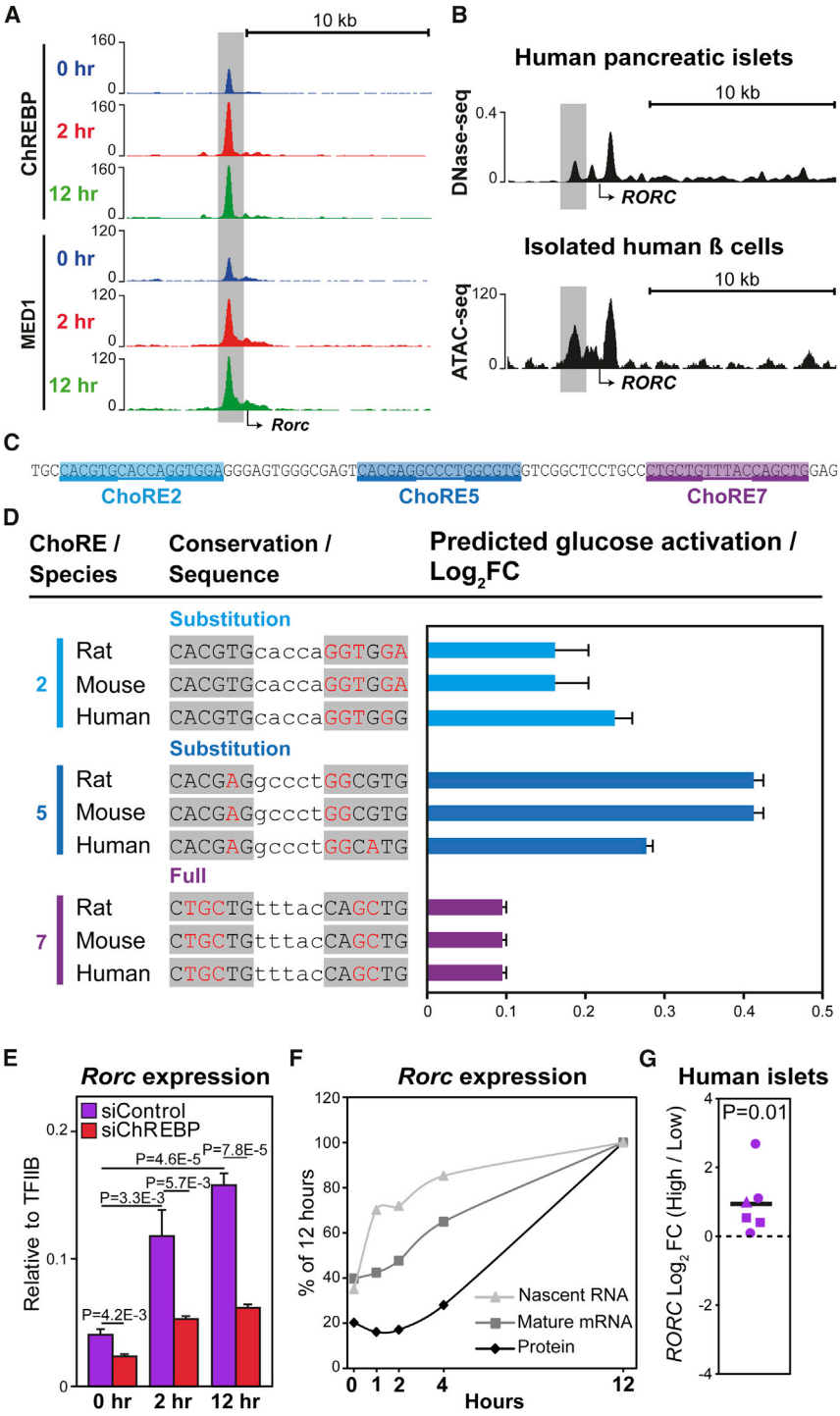


Figure 5. RORγ Is a ChREBP Target in β Cells

(A) UCSC Genome Browser screenshots of MED1- and ChREBP-occupancy at the *Rorc* locus in INS-1E cells treated with 25 mM glucose for 0 hr, 2 hr, and 12 hr. The gray bar marks the identified ChREBP-bound *Rorc* −1 kb enhancer.

(B) UCSC Genome Browser screenshots displaying DNase-seq signal at the *RORC* locus in human pancreatic islets (Encode, 2012) (B, top) and ATAC-seq signal in isolated primary human β cells (Ackermann et al., 2016) (B, bottom). The gray bars mark the orthologous region corresponding to the rat *Rorc* −1 kb enhancer.

(C) Sequence of the central rat *Rorc* −1 kb enhancer. The three non-overlapping putative ChoREs (2, 5, and 7) with the highest predicted combined activity are depicted. See also Figures S5A and S5B.

(D) Conservation and predicted activities of ChoRE2, ChoRE5, and ChoRE7 in the mouse and human genomes. The activities were predicted based on the number of mismatches to the primary and secondary E-box as described in Figures 3G, S3G, and S3H. See also Figure S5B. The bars indicate mean and 95% confidence intervals of glucose activation (log_2 fold change [MED1 2 hr / MED1 0 hr]).

(E) Bar diagram showing nascent *Rorc* transcript levels in siChREBP or siControl transfected cells treated with high glucose for 0, 2, or 12 hr.

(F) Nascent (light gray) and mature (dark gray) transcript levels of *Rorc* and protein levels of RORγ (black) in INS-1E cells treated with 25 mM glucose for 0, 1, 2, 4, and 12 hr. The percentages relative to the level at 12 hr are plotted.

(G) Dot blots representing glucose-induced log_2 fold changes from all six individual donors in the three studies analyzed in Figure S5D. The squares represent (Brun et al., 2015) (Figure S5D, left), the circles represent (Bagge et al., 2012) (Figure S5D, center), the triangle represents (Boergesen et al., 2011) (Figure S5D, right), and the black bar represents the average of all samples. The indicated p value was calculated using a one-tailed Student's t test.

factors, RFX3 and FOXO1 are particularly interesting, as RFX3 is required for proper β cell differentiation and function (Ait-Lounis et al., 2010), and β cell specific FOXO1 ablation in mice leads to increased susceptibility to β cell dedifferentiation by metabolic stress (Talchai et al., 2012). As several of the identified TFs are known to regulate the expression of each other, we further hypothesize that the acute repression of TFs like RFX3 and FOXO1 initiates an amplifying feedforward mechanism eventually leading to the delayed full repression of β cell identity genes including genes in the MODY and insulin secretion pathways. Importantly, from these analyses, several potential regulators of β cell identity emerge, including HMX1, HOXC13, and BARHL1. The role of these in β cell development, function, and dedifferentiation should be further investigated.

Similarly, the rapid induction of ChREBP binding and the limited enrichment hereof at late-activated enhancers, indicate that ChREBP mediate the late effects of glucose through induction of secondary transcriptional regulators, the accumulation of

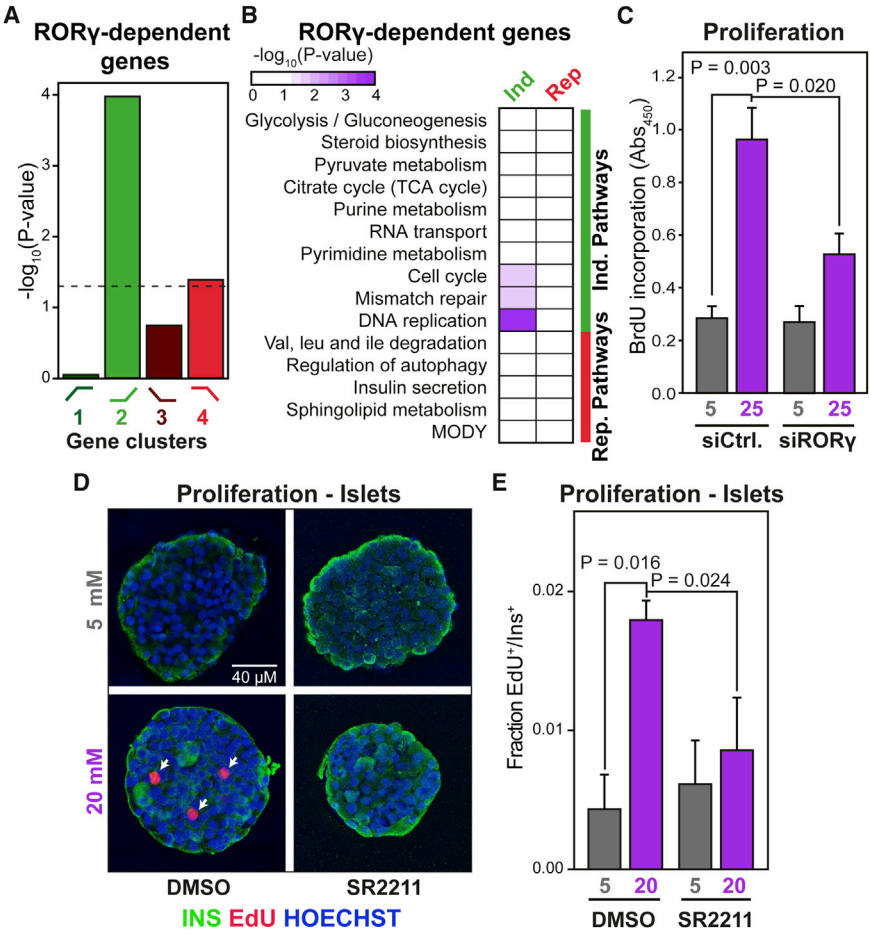


Figure 6. RORγ Is Required for Glucose-Induced β Cell Proliferation

(A) Bar diagram illustrating the overlap between RORγ-dependent genes and the four gene clusters defined in Figure 1C. RORγ dependent genes were defined by a one-sided Student's t test between log_2 fold changes in siControl and siRORγ and the significance in a one-sided chi-square test of the overlap between the list of RORγ-dependent genes and the four gene clusters defined in Figure 1C compared with constitutive genes was calculated.

(B) Heatmap illustrating pathway enrichment of genes differentially regulated in siRORγ- versus siControl-transfected cells.

(C) Bar diagram representing proliferation of INS-1E cells in siControl- and siRORγ-transfected cells. The cells were transfected with siRNA and 48 hr later preincubated in 5 mM glucose for 24 hr followed by 24 hr exposure to 5 or 25 mM glucose. BrdU was added 6 hr prior to harvest and subsequently quantified by ELISA. The bars represent mean and SEM of three independent experiments.

(D and E) Pancreatic islets isolated from adult rats were seeded onto coverslips and cultured for 48 hr in 5 or 20 mM glucose medium supplemented with RORγ antagonist SR2211 (20 μM) or DMSO, and β cell proliferation was assessed by EdU incorporation.

(D) Representative immunocytochemical staining of rat pancreatic islets cultured with glucose and SR2211 as indicated and stained for EdU (red), insulin (green), and DNA/Hoechst (blue).

(E) Bar diagrams representing the fractions of EdU+/Ins+ positive cells in pancreatic islets cultured with glucose and SR2211 as indicated. The bars represent mean and SEM of 3–4 independent biological experiments. The indicated p values were calculated using a one-tailed Student's t test (C and E).

which results in activation of the second wave gene program. Interestingly, the Scott laboratory recently showed that induction of ChREBPβ, a truncated constitutive active isoform of ChREBP which is induced by ChREBP itself (Herman et al., 2012), is required for glucose-induced β cell proliferation (Zhang et al., 2015). The finding that ChREBPβ is a ChREBPα target gene suggests that it could activate second wave enhancers; however, since ChREBP binding sites are not enriched in these enhancers, we consider it more likely that ChREBPβ amplifies the ChREBPα activity in the first wave enhancers. By applying our integrative approach to late activated enhancers, we identified 53 enriched motifs for which 13 of the corresponding TFs are transcriptionally activated by glucose. Whereas the function and role of multiple of the potential regulators of late repressed enhancers and β cell identity genes are well characterized, the potential of the identified late activators have not been described in the context of β cell proliferation. Furthermore, with MYCN as the exception, little is known about the role of these factors in cell proliferation in general. We therefore investigated these factors by siRNA-mediated knockdown and confirmed a role for MYCN, ATF4, GATA4, and RORγ in the induction of cell-cycle genes by glucose in INS-1E β cells. We propose that these ChREBP-induced factors cooperate to mediate the full induction of cell-cycle genes and β cell proliferation by glucose. Further investigations of these ChREBP-induced secondary regulators of β cell proliferation may outline opportunities for specific modulation of β cell proliferation in T2D therapy.

We chose to focus on the nuclear receptor RORγ for further investigations, and we demonstrated that the *Rorc* gene encoding RORγ is a direct target of ChREBP and acutely activated by glucose. Further analyses of RNA-seq data from RORγ-depleted cells indicated that RORγ is selectively involved in regulation of the late induced gene program, including cell-cycle genes, by glucose. Importantly, RORγ activity is required for full glucose-induced proliferation of both INS-1E cells and β cells within primary rat islets cultured ex vivo, further indicating a positive role of RORγ in β cell adaptation in response to metabolic stress. Such a role may seem contradictory to the phenotype of RORγ−/− mice, which exhibit improved insulin sensitivity and glucose tolerance on a high fat diet (Meissburger et al., 2011; Takeda et al., 2014); however, these effects appear to be due to RORγ action in adipose tissue (Meissburger et al., 2011) and liver (Takeda et al., 2014). Future studies should investigate the β cell-specific role

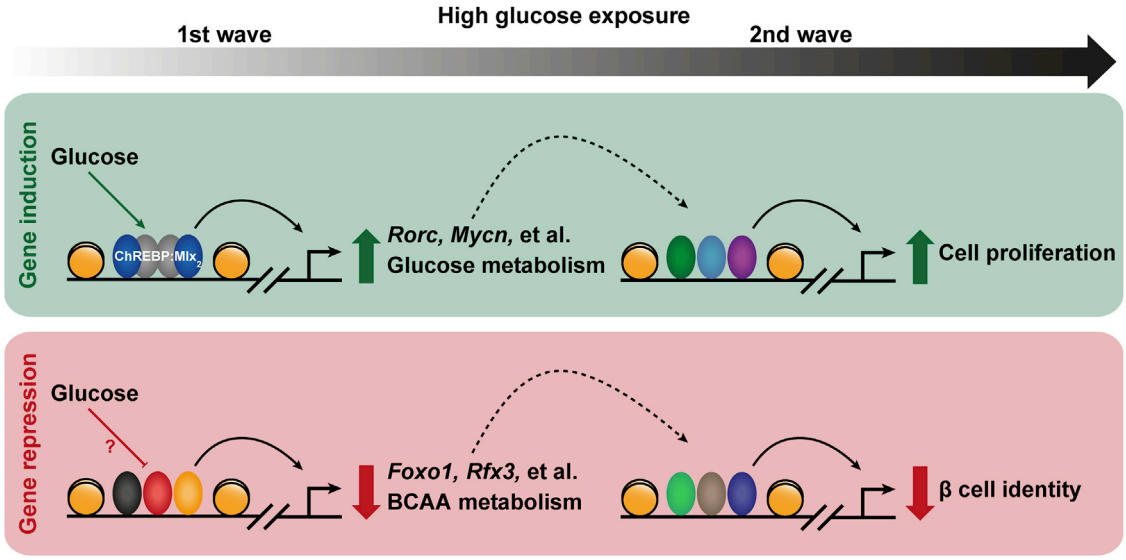


Figure 7. Model of Glucose-Induced, ChREBP-Mediated Reprogramming of the β Cell Enhancer and Gene Landscape

Following glucose treatment, ChREBP activates genes encoding metabolic enzymes and induces the expression of a set of transcriptional regulators including RORγ, the accumulation of which results in induction of the cell-cycle gene program. Additionally, and possibly by ChREBP-mediated cofactor redistribution, glucose treatment acutely leads to repression of genes encoding transcriptional regulators like RFX3 and FOXO1, the downregulation of which may facilitate the delayed repression of β cell identity genes.

of RORγ in response to metabolic stress and explore the ability of RORγ agonists to promote β cell proliferation in vivo. From a therapeutic perspective, it is promising that short term administration of a dual RORα/γ agonist was recently shown to improve glucose tolerance in mice, although this was suggested to be facilitated by a RORα-mediated increase in insulin expression (Kuang et al., 2014). Conversely, potential undesired effects of RORγ antagonists on β cell proliferation should be considered when exploring the use of these antagonists as potential insulin-sensitizing drugs due to their inhibition of RORγ in liver and adipose tissue. It will also be important to define the mechanism of RORγ action including the identification of the direct target genes of RORγ and to determine the importance of crosstalk with the other cell-cycle regulators induced by glucose.

In conclusion, our work shows that high glucose leads to a biphasic change in gene expression in INS-1E cells, where cell-cycle genes are activated, and β cell genes are repressed in the second wave (Figure 7). ChREBP acts as a master regulator of the transcriptional response to glucose, but is primarily directly associated with enhancers activated in the first wave, whereas enhancers activated in the second wave appear to be activated by ChREBP-induced TFs. These include RORγ, which we identify as a regulator of glucose-induced β cell proliferation.

EXPERIMENTAL PROCEDURES

Cell Cultures

The INS-1E cell line (Merglen et al., 2004) was cultured as previously described (Boergesen et al., 2011) and used between passage numbers 60 and 90. Prior to exposure to high (25 mM) glucose, cells were preincubated with 5 mM glucose medium for 24 hr. Time course experiments were performed in a reverse manner, i.e., medium was changed on all cells at time point 0 hr, whereafter 25 mM glucose was added to the medium at consecutive time points and all cells were harvested at the 12 hr time point.

Pancreatic islets were isolated from adult rats (8- to 10-week-old Sprague-Dawley) by collagenase infusion via the common bile duct as previously described (Roeske-Nielsen et al., 2010). For proliferation experiments, islets were cultured for 72 hr in RPMI 1640 supplemented with 10% FCS and 1% penicillin-streptomycin with media change daily, before seeding overnight onto Matrigel-coated (Corning) coverslips (10–30 islets per coverslip) in RPMI 1640 supplemented with 2% pooled human serum (MP Cellect) and 1% penicillin-streptomycin. The care of rats was within Institutional Animal Care and Use Committee (IACUC) guidelines. Analysis of *RORC* expression in human islets in Figures 5G and S5D was performed on material previously collected, and detailed information on isolation, culture, and treatment of human islets for the experiments can be found in (Bagge et al., 2012; Boergesen et al., 2011; Brun et al., 2015). The use of human tissue for research was approved by the local ethical committees.

RNA Extraction, cDNA Synthesis, and Quantitative Real-Time PCR

Following Isol-RNA lysis Reagent (5-Prime) extraction and EconoSpin (Epoc Life) column purification of total RNA, cDNA synthesis, and quantitative real-time PCR (qPCR) were performed as previously described (Boergesen et al., 2011). Sequences of primers used for qPCR are available in Table S4.

RNA-Seq

For total RNA-seq, contaminant genomic DNA was removed from purified total RNA by TURBO DNase digestion (Life Technologies), and rRNAs were removed using the Ribo-Zero Human/Mouse/Rat kit (Epicenter). For mRNA-seq, 1 μg total RNA was incubated with poly-dT beads to isolate polyadenylated RNA. RNA fragmentation and cDNA synthesis was performed according to the manufacturer's instructions (TruSeq 2, Illumina).

ChIP-Seq

ChIP experiments were performed according to standard protocol as described (Siersbæk et al., 2012). Antibodies used were ChREBP (NB400-135, Novus Biologicals) and MED1 (M-255, sc-8998, Santa Cruz).

DNase-Seq

DNase-seq was performed on ~10 million nuclei essentially as previously described (Siersbæk et al., 2011).

Library Construction and Sequencing

RNA-, DNase-, and ChIP-seq libraries were constructed using PentAdapters (Pentabase) essentially as described in Nielsen and Mandrup (2014). Sequencing was performed on the Illumina HiSeq 1500 platform.

Adenoviral Transduction

The CA-ChREBP adenovirus used was generated and produced as previously described (Boergesen et al., 2011). INS-1E cells were preincubated for 24 hr, incubated with equal titers of CA-ChREBP or GFP control adenovirus for 2 hr, and incubated with 5 mM glucose for 12 hr before harvest of RNA.

siRNA-Mediated Knockdown

At 1 day prior to transfection, INS-1E cells were seeded in antibiotic-free culture medium at a density of 800,000 cells/cm^2. A transfection mix (750 nM siRNA [Sigma-Aldrich] and 5% v/v DharmaFECT [Thermo]) prepared in serum- and antibiotic-free OptiMEM media (Gibco) was added to the cells in a 1:5 ratio. The following day, the media was changed to standard RPMI, and the cells were incubated for 2 days prior to any experiment. Oligo sequences are available in Table S4.

Western Blotting and ECL Detection

Whole cell extracts were prepared in SDS-containing buffer and subjected to western blotting as previously described (Nielsen et al., 2006). The following primary antibodies were used: anti-ChREBP (sc-21189, Santa Cruz Biotechnology) and anti-RORγ (MAB6109; R&D systems). Anti-HSP90 (sc-7947, Santa-Cruz Biotechnology) or anti-TFIIB (sc-225, Santa Cruz Biotechnology) was included as loading controls. The following secondary antibodies were used: anti-rabbit IgG (P0399, Dako) and anti-mouse IgG (P0447, DAKO). Densitometry was used to quantify protein expression.

Cell Proliferation Assays

Proliferation of INS-1E cells was determined by BrdU incorporation. Briefly, cells were preincubated for 24 hr in 5 mM glucose medium and then stimulated with either 5 mM glucose or 25 mM glucose for 24 hr. BrdU was added to the medium for the last 6 hr before the cells were fixed. Following DNA denaturation, the amount of BrdU incorporation was assessed immunochemically as described in the manufacturer's (Millipore) protocol.

Proliferation of primary rat β cells was assessed by EdU-incorporation in intact rat pancreatic cultured ex vivo essentially as described in Bruun et al. (2014). For investigation of glucose-stimulated β cell proliferation, medium was changed to either 5 mM or 20 mM glucose medium supplemented with 20 μM SR2211 (Millipore) or DMSO for 48 hr. EdU was supplemented to all conditions for the full 48 hr at a concentration of 10 μM (Click-IT EdU, Invitrogen). Following stimulation, islets were fixed in 4% paraformaldehyde, washed in PBS, and permeabilized in 17% DMSO before EdU detection and stained for insulin using guinea pig anti-insulin (Ab7842, Abcam) followed by visualization using Dylight488 conjugated goat-anti-guinea pig (Ab102374, Abcam). Hoechst 33342 was used for nuclear counterstaining. z stack images were captured by confocal microscopy (Zeiss LSM 510) and counted using Fiji (http://www.fiji.sc/). A minimum of 3,500 Ins$^+$ cells were counted in total per condition.

Additional Data Sets

The following publically available data sets were used in this work: RNA-seq data from the rat body map (Yu et al., 2014) (GEO: GSE53960), microarray data from glucose treatment of rat pancreatic islets (Bensellam et al., 2009) (GEO: GSE12817), microarray data from pancreatic islets of human type 2 diabetic and control subjects (Taneera et al., 2012) (GEO: GSE38642), DNase-seq data from human pancreatic islets (Encode, 2012) (GEO: GSM816660), and ATAC-seq data from isolated human primary β cells (Ackermann et al., 2016) (GEO: GSE76268).

Statistical Analysis

Bar diagrams illustrating qPCR or proliferation analyses are represented as mean + SE. The indicated p values were calculated using a one-tailed Student's t test. RNA- and ChIP-seq data were analyzed for differential expression or occupancy using iRNA-seq (Madsen et al., 2015) and edgeR (Robinson et al., 2010), respectively. Cluster analyses were performed using fuzzy c-means clustering (Maechler et al., 2016). Differences in expression strength or occupancy between gene and enhancer groups were tested using non-parametric Wilcoxon ranked-sum tests. The significance of any enrichments or overlaps was determined by random permutation, chi-square tests, or linear regression as indicated in the figures.

Detailed descriptions of the computational analyses used in this study are available in the Supplemental Information.

ACCESSION NUMBERS

The accession number for the sequencing data sets reported in this paper is GEO: GSE81628.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, six figures, and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.07.063.

AUTHOR CONTRIBUTIONS

S.F.S., J.G.S.M., and S.M. conceived and designed the study. S.F.S., J.G.S.M., K.Ø.F., L.L.C.P., S.S., M.B., A.L., B.D.L., M.S.M., and L.T.D. performed the experiments. J.G.S.M. analyzed the data with input from S.F.S., P.M. provided material, J.J.H. supported the stipend of J.G.S.M., and L.T.D. and S.M. supervised the study. J.G.S.M. and S.F.S. prepared the figures and S.F.S. and S.M. wrote the manuscript with input from the other authors.

ACKNOWLEDGMENTS

The authors thank members of the Mandrup laboratory, in particular Rasmus Siersbæk and Ronni Nielsen, for experimental support and fruitful discussions. We also thank Christa Persson, Roskilde University for technical assistance with assessment of β cell proliferation in primary rat islets and Dominique Duhamel (University of Geneva) for experiments on human islets. Finally, we thank Jonathan Brewer and Jes Dreier, DaMBIC, University of Southern Denmark for technical assistance with bioimaging experiments.

This work was supported by grants from the Novo Nordisk Foundation (to S.M., L.T.D., and J.J.H.), the Danish Independent Research Council | Health Sciences (to S.M. and L.T.D.), the European Foundation for the Study of Diabetes (to S.M.), the Swiss National Science Foundation (to P.M.), and research grant from the Danish Diabetes Academy supported by the Novo Nordisk Foundation (to S.S.). Furthermore, the work was supported by grants from NordForsk to the Nordic Center of Excellence MitoHealth, from the Danish Agency for Science Technology and Innovation to the DaMBIC bioimaging research core facility at the University of Southern Denmark, and from the VILLUM Foundation to the VILLUM Center for Bioanalytical Sciences at University of Southern Denmark.

We acknowledge the use of data from the ENCODE (Crawford lab) and Beta Cell Biology Consortia. Human islets were provided through the Juvenile Diabetes Research Foundation (JDRF) islet redistribution program by Islet Cell Resource Centers in Milan, Uppsala, and Geneva (award 1-RSC-2014-100-I-X [ECIT Islet for Basic Research program]).

Received: February 29, 2016
Revised: May 26, 2016
Accepted: July 25, 2016
Published: August 18, 2016

REFERENCES

Ackermann, A.M., Wang, Z., Schug, J., Naji, A., and Kaestner, K.H. (2016). Integration of ATAC-seq and RNA-seq identifies human alpha cell and beta cell signature genes. Mol. Metab. *5*, 233–244.

Aguayo-Mazzucato, C., Zavacki, A.M., Marinelarena, A., Hollister-Lock, J., El Khattabi, I., Marsili, A., Weir, G.C., Sharma, A., Larsen, P.R., and Bonner-Weir, S. (2013). Thyroid hormone promotes postnatal rat pancreatic β cell development and glucose-responsive insulin secretion through MAFA. Diabetes *62*, 1569–1580.

Ait-Lounis, A., Bonal, C., Seguín-Estévez, Q., Schmid, C.D., Bucher, P., Herrera, P.L., Durand, B., Meda, P., and Reith, W. (2010). The transcription factor Rfx3 regulates beta cell differentiation, function, and glucokinase expression. Diabetes *59*, 1674–1685.

Bagge, A., Clausen, T.R., Larsen, S., Ladefoged, M., Rosenstierne, M.W., Larsen, L., Vang, O., Nielsen, J.H., and Dalgaard, L.T. (2012). MicroRNA-29a is up-regulated in beta cells by glucose and decreases glucose-stimulated insulin secretion. Biochem. Biophys. Res. Commun. *426*, 266–272.

Barbagallo, D., Condorelli, A.G., Piro, S., Parrinello, N., Fløyel, T., Ragusa, M., Rabuazzo, A.M., Størling, J., Purrello, F., Di Pietro, C., and Purrello, M. (2014). CEBPA exerts a specific and biologically important proapoptotic role in pancreatic β cells through its downstream network targets. Mol. Biol. Cell *25*, 2333–2341.

Bell, E., Chen, L., Liu, T., Marshall, G.M., Lunec, J., and Tweddle, D.A. (2010). MYCN oncoprotein targets and their therapeutic potential. Cancer Lett. *293*, 144–157.

Bensellam, M., Van Lommel, L., Overbergh, L., Schuit, F.C., and Jonas, J.C. (2009). Cluster analysis of rat pancreatic islet gene mRNA levels after culture in low-, intermediate- and high-glucose concentrations. Diabetologia *52*, 463–476.

Boergesen, M., Poulsen, LI., Schmidt, S.F., Frigerio, F., Maechler, P., and Mandrup, S. (2011). ChREBP mediates glucose repression of peroxisome proliferator-activated receptor alpha expression in pancreatic beta cells. J. Biol. Chem. *286*, 13214–13225.

Brun, T., Li, N., Jourdain, A.A., Gaudet, P., Duhamel, D., Meyer, J., Bosco, D., and Maechler, P. (2015). Diabetogenic milieus induce specific changes in mitochondrial transcriptome and differentiation of human pancreatic islets. Hum. Mol. Genet. *24*, 5270–5284.

Bruun, C., Christensen, G.L., Jacobsen, M.L., Kanstrup, M.B., Jensen, P.R., Fjordvang, H., Mandrup-Poulsen, T., and Billestrup, N. (2014). Inhibition of beta cell growth and function by bone morphogenetic proteins. Diabetologia *57*, 2546–2554.

Cairo, S., Merla, G., Urbinati, F., Ballabio, A., and Reymond, A. (2001). WBSCR14, a gene mapping to the Williams–Beuren syndrome deleted region, is a new member of the Mlx transcription factor network. Hum. Mol. Genet. *10*, 617–627.

Crawford, G.E., Holt, I.E., Whittle, J., Webb, B.D., Tai, D., Davis, S., Margulies, E.H., Chen, Y., Bernat, J.A., Ginsburg, D., et al. (2006). Genome-wide mapping of DNase hypersensitive sites using massively parallel signature sequencing (MPSS). Genome Res. *16*, 123–131.

da Silva Xavier, G., Rutter, G.A., Diraison, F., Andreolas, C., and Leclerc, I. (2006). ChREBP binding to fatty acid synthase and L-type pyruvate kinase genes is stimulated by glucose in pancreatic beta cells. J. Lipid Res. *47*, 2482–2491.

Doyle, M.J., and Sussel, L. (2007). Nkx2.2 regulates beta cell function in the mature islet. Diabetes *56*, 1999–2007.

Encode (2012). An integrated encyclopedia of DNA elements in the human genome. Nature *489*, 57–74.

Harrison, K.A., Thaler, J., Pfaff, S.L., Gu, H., and Kehrl, J.H. (1999). Pancreas dorsal lobe agenesis and abnormal islets of Langerhans in Hlxb9-deficient mice. Nat. Genet. *23*, 71–75.

Heintzman, N.D., Hon, G.C., Hawkins, R.D., Kheradpour, P., Stark, A., Harp, L.F., Ye, Z., Lee, L.K., Stuart, R.K., Ching, C.W., et al. (2009). Histone modifications at human enhancers reflect global cell-type-specific gene expression. Nature *459*, 108–112.

Heit, J.J., Apelqvist, A.A., Gu, X., Winslow, M.M., Neilson, J.R., Crabtree, G.R., and Kim, S.K. (2006). Calcineurin/NFAT signalling regulates pancreatic beta cell growth and function. Nature *443*, 345–349.

Herman, M.A., Peroni, O.D., Villoria, J., Schön, M.R., Abumrad, N.A., Blüher, M., Klein, S., and Kahn, B.B. (2012). A novel ChREBP isoform in adipose tissue regulates systemic glucose metabolism. Nature *484*, 333–338.

Holland, A.M., Góñez, L.J., Naselli, G., Macdonald, R.J., and Harrison, L.C. (2005). Conditional expression demonstrates the role of the homeodomain transcription factor Pdx1 in maintenance and regeneration of beta cells in the adult pancreas. Diabetes *54*, 2586–2595.

Hurst, L.D., Sachenkova, O., Daub, C., Forrest, A.R., and Huminiecki, L.; FANTOM consortium (2014). A simple metric of promoter architecture robustly predicts expression breadth of human genes suggesting that most transcription factors are positive regulators. Genome Biol. *15*, 413.

Jackerott, M., Møldrup, A., Thams, P., Galsgaard, E.D., Knudsen, J., Lee, Y.C., and Nielsen, J.H. (2006). STAT5 activity in pancreatic beta cells influences the severity of diabetes in animal models of type 1 and 2 diabetes. Diabetes *55*, 2705–2712.

Jeong, Y.S., Kim, D., Lee, Y.S., Kim, H.J., Han, J.Y., Im, S.S., Chong, H.K., Kwon, J.K., Cho, Y.H., Kim, W.K., et al. (2011). Integrated expression profiling and genome-wide analysis of ChREBP targets reveals the dual role for ChREBP in glucose-regulated gene expression. PLoS ONE *6*, e22544.

Kim, T., Hemberg, M., Gray, J., Costa, A., Bear, D., Wu, J., Harmin, D., Laptewicz, M., Barbara-Haley, K., Kuersten, S., et al. (2010). Widespread transcription at neuronal activity-regulated enhancers. Nature *465*, 7182–7297.

Kitamura, T. (2013). The role of FOXO1 in β cell failure and type 2 diabetes mellitus. Nat. Rev. Endocrinol. *9*, 615–623.

Kuang, J., Hou, X., Zhang, J., Chen, Y., and Su, Z. (2014). Identification of insulin as a novel retinoic acid receptor-related orphan receptor α target gene. FEBS Lett. *588*, 1071–1079.

Levitt, H.E., Cyphert, T.J., Pascoe, J.L., Hollern, D.A., Abraham, N., Lundell, R.J., Rosa, T., Romano, L.C., Zou, B., O'Donnell, C.P., et al. (2011). Glucose stimulates human beta cell replication in vivo in islets transplanted into NOD-severe combined immunodeficiency (SCID) mice. Diabetologia *54*, 572–582.

Li, M.V., Chang, B., Imamura, M., Poungvarin, N., and Chan, L. (2006). Glucose-dependent transcriptional regulation by an evolutionarily conserved glucose-sensing module. Diabetes *55*, 1179–1189.

Lingohr, M.K., Dickson, L.M., McCuaig, J.F., Hugl, S.R., Twardzik, D.R., and Rhodes, C.J. (2002). Activation of IRS-2-mediated signal transduction by IGF-1, but not TGF-alpha or EGF, augments pancreatic beta cell proliferation. Diabetes *51*, 966–976.

Ma, L., Tsatsos, N.G., and Towle, H.C. (2005). Direct role of ChREBP.Mlx in regulating hepatic glucose-responsive genes. J. Biol. Chem. *280*, 12019–12027.

Ma, L., Robinson, L.N., and Towle, H.C. (2006). ChREBP*Mlx is the principal mediator of glucose-induced gene expression in the liver. J. Biol. Chem. *281*, 28721–28730.

Ma, L., Sham, Y.Y., Walters, K.J., and Towle, H.C. (2007). A critical role for the loop region of the basic helix-loop-helix/leucine zipper protein Mlx in DNA binding and glucose-regulated transcription. Nucleic Acids Res. *35*, 35–44.

Madsen, J.G., Schmidt, S.F., Larsen, B.D., Loft, A., Nielsen, R., and Mandrup, S. (2015). iRNA-seq: computational method for genome-wide assessment of acute transcriptional regulation from total RNA-seq data. Nucleic Acids Res. *43*, e40.

Maechler, M., Rousseeuw, P., Struyf, A., Hubert, M., and Hornik, K. (2016). cluster: Cluster Analysis Basics and Extensions. R package version, 2.0.4.

Meissburger, B., Ukropec, J., Roeder, E., Beaton, N., Geiger, M., Teupser, D., Civan, B., Langhans, W., Nawroth, P.P., Gasperikova, D., et al. (2011). Adipogenesis and insulin sensitivity in obesity are regulated by retinoid-related orphan receptor gamma. EMBO Mol. Med. *3*, 637–651.

Merglen, A., Theander, S., Rubi, B., Chaffard, G., Wollheim, C.B., and Maechler, P. (2004). Glucose sensitivity and metabolism-secretion coupling studied during two-year continuous culture in INS-1E insulinoma cells. Endocrinology *145*, 667–678.

Metukuri, M.R., Zhang, P., Basantani, M.K., Chin, C., Stamateris, R.E., Alonso, L.C., Takane, K.K., Gramignoli, R., Strom, S.C., O'Doherty, R.M., et al. (2012). ChREBP mediates glucose-stimulated pancreatic β cell proliferation. Diabetes *61*, 2004–2015.

Moore, F., Naamane, N., Colli, M.L., Bouckenooghe, T., Ortis, F., Gurzov, E.N., Igoillo-Esteve, M., Mathieu, C., Bontempi, G., Thykjaer, T., et al. (2011). STAT1 is a master regulator of pancreatic beta cell apoptosis and islet inflammation. J. Biol. Chem. *286*, 929–941.

Nielsen, R., and Mandrup, S. (2014). Genome-wide profiling of transcription factor binding and epigenetic marks in adipocytes by ChIP-seq. Methods Enzymol. *537*, 261–279.

Nielsen, R., Grøntved, L., Stunnenberg, H.G., and Mandrup, S. (2006). Peroxisome proliferator-activated receptor subtype- and cell-type-specific activation of genomic target genes upon adenoviral transgene delivery. Mol. Cell. Biol. *26*, 5698–5714.

Ohta, Y., Kosaka, Y., Kishimoto, N., Wang, J., Smith, S.B., Honig, G., Kim, H., Gasa, R.M., Neubauer, N., Liou, A., et al. (2011). Convergence of the insulin and serotonin programs in the pancreatic β cell. Diabetes *60*, 3208–3216.

Poungvarin, N., Lee, J.K., Yechoor, V.K., Li, M.V., Assavapokee, T., Suksaranjit, P., Thepsongwajja, J.J., Saha, P.K., Oka, K., and Chan, L. (2012). Carbohydrate response element-binding protein (ChREBP) plays a pivotal role in beta cell glucotoxicity. Diabetologia *55*, 1783–1796.

Poungvarin, N., Chang, B., Imamura, M., Chen, J., Moolsuwan, K., Sae-Lee, C., Li, W., and Chan, L. (2015). Genome-wide analysis of ChREBP binding sites on male mouse liver and white adipose chromatin. Endocrinology *156*, 1982–1994.

Prentki, M., and Nolan, C.J. (2006). Islet beta cell failure in type 2 diabetes. J. Clin. Invest. *116*, 1802–1812.

Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics *26*, 139–140.

Roeske-Nielsen, A., Dalgaard, L.T., Månsson, J.E., and Buschard, K. (2010). The glycolipid sulfatide protects insulin-producing cells against cytokine-induced apoptosis, a possible role in diabetes. Diabetes Metab. Res. Rev. *26*, 631–638.

Schmidt, S.F., Larsen, B.D., Loft, A., Nielsen, R., Madsen, J.G., and Mandrup, S. (2015). Acute TNF-induced repression of cell identity genes is mediated by NFκB-directed redistribution of cofactors from super-enhancers. Genome Res. *25*, 1281–1294.

Schmidt, S.F., Larsen, B.D., Loft, A., and Mandrup, S. (2016). Cofactor squelching: Artifact or fact? BioEssays *38*, 618–626.

Siersbæk, R., Nielsen, R., John, S., Sung, M.H., Baek, S., Loft, A., Hager, G.L., and Mandrup, S. (2011). Extensive chromatin remodelling and establishment of transcription factor 'hotspots' during early adipogenesis. EMBO J. *30*, 1459–1472.

Siersbæk, M.S., Loft, A., Aagaard, M.M., Nielsen, R., Schmidt, S.F., Petrovic, N., Nedergaard, J., and Mandrup, S. (2012). Genome-wide profiling of peroxisome proliferator-activated receptor γ in primary epididymal, inguinal, and brown adipocytes reveals depot-selective binding correlated with gene expression. Mol. Cell. Biol. *32*, 3452–3463.

Stoeckman, A.K., Ma, L., and Towle, H.C. (2004). Mlx is the functional heteromeric partner of the carbohydrate response element-binding protein in glucose regulation of lipogenic enzyme genes. J. Biol. Chem. *279*, 15662–15669.

Takeda, Y., Kang, H.S., Freudenberg, J., DeGraff, L.M., Jothi, R., and Jetten, A.M. (2014). Retinoic acid-related orphan receptor γ (RORγ): a novel participant in the diurnal regulation of hepatic gluconeogenesis and insulin sensitivity. PLoS Genet. *10*, e1004331.

Talchai, C., Xuan, S., Lin, H.V., Sussel, L., and Accili, D. (2012). Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure. Cell *150*, 1223–1234.

Taneera, J., Lang, S., Sharma, A., Fadista, J., Zhou, Y., Ahlqvist, E., Jonsson, A., Lyssenko, V., Vikman, P., Hansson, O., et al. (2012). A systems genetics approach identifies genes and pathways for type 2 diabetes in human islets. Cell Metab. *16*, 122–134.

Yu, Y., Fuscoe, J.C., Zhao, C., Guo, C., Jia, M., Qing, T., Bannon, D.I., Lancashire, L., Bao, W., Du, T., et al. (2014). A rat RNA-Seq transcriptomic BodyMap across 11 organs and 4 developmental stages. Nat. Commun. *5*, 3230.

Zhang, P., Kumar, A., Katz, L.S., Li, L., Paulynice, M., Herman, M.A., and Scott, D.K. (2015). Induction of the ChREBPbeta isoform is essential for glucose-stimulated beta cell proliferation. Diabetes *64*, 4158–4170.

Zitzer, H., Wente, W., Brenner, M.B., Sewing, S., Buschard, K., Gromada, J., and Efanov, A.M. (2006). Sterol regulatory element-binding protein 1 mediates liver X receptor-beta-induced increases in insulin secretion and insulin messenger ribonucleic acid levels. Endocrinology *147*, 3898–3905.