CHARACTERIZATION OF PANCREATIC TRANSCRIPTION FACTOR PDX-1 BINDING SITES USING PROMOTER MICROARRAY AND SERIAL ANALYSIS OF CHROMATIN OCCUPANCY

David M. Keller¹, Shannon McWeeney², Athanasios Arsenlis³, Jacques Drouin⁴, Christopher V.E. Wright⁵, Haiyan Wang⁶, Claes B. Wollheim⁷, Peter White³, Klaus H. Kaestner³, and Richard H. Goodman¹

¹Vollum Institute, and ²Division of Biostatistics, Department of Public Health and Preventative Medicine, Oregon Health & Science University, Portland, OR 97239 USA, ³Department of Genetics, and Institute for Diabetes, Obesity, and Metabolism, University of Pennsylvania, Philadelphia, PA, 19104 USA, ⁴Institut de Recherches Cliniques de Montreal, Montreal, Quebec H2W 1R7 Canada, ⁵Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN 37232 USA, ⁶F. Hoffmann-La Roche Ltd, Basel, Switzerland, ⁷Department of Cell Physiology and Metabolism, University Medical Center, Geneva, Switzerland

Running title: Identification of Pdx-1 binding sites

Address correspondence to: David M. Keller, Vollum Institute, OHSU, 3181 SW Sam Jackson Park Rd., Portland, OR 97239, Tel. 503-494-4676; Fax. 503-494-4353; E-mail:kellerda@hotmail.com

The homeobox transcription factor Pdx-1 is necessary for pancreas organogenesis and beta cell function, however most Pdx-1 regulated genes are unknown. To further the understanding of Pdx-1 in beta cell biology, we have characterized its genomic targets in NIT-1 cells, a mouse insulinoma cell line. To identify novel targets, we developed a microarray that includes traditional promoters as well as non-coding conserved elements, microRNAs, and elements identified through an unbiased approach termed Serial Analysis of Chromatin Occupancy (SACO). In total, 583 new Pdx-1 target genes were identified, many of which contribute to energy sensing and insulin release in pancreatic beta cells. By analyzing 31 of the protein-coding Pdx-1 target genes, we show that 29 are expressed in beta cells and, of these, 68% are down- or up-regulated in cells expressing a dominant negative mutant of Pdx-1. We additionally show that many Pdx-1 targets also interact with NeuroD1/BETA2, including the microRNA miR-375, a known regulator of insulin secretion.

Nearly 800,000 new cases of diabetes mellitus are diagnosed every year in the U.S. Although insulin replacement remains the mainstay of treatment, considerable effort has been devoted to developing approaches for cell-based therapy (1). Through these efforts, it may become possible to introduce cells into diabetic patients that not only produce insulin, but also respond appropriately to metabolic signals that regulate insulin production, processing, and secretion. Accomplishing this goal demands a better understanding of the essential determinants of the beta cell phenotype. While no single transcription factor can be considered to be the sole determinant of beta cell function, the homeodomain-containing factor Pdx-1 (Ipfl/Idx-1/Stf-1/MODY4) has been shown to be a major regulator of beta cell function. Initially characterized because of its role in regulating the insulin and somatostatin promoters (2-4), Pdx-1 is now known to be critical for many aspects of pancreatic development (5-9). Indeed, Pdx-1 is being tested for its ability to generate beta cells from embryonic stem (ES) (10) and liver cells (11-15) for use in beta cell transplants. Additionally,
Pdx-1 is expressed in adult beta cells and thus is a potential target for therapeutics. In fact, a newly approved anti-diabetic drug, exendin-4, functions in part through an intracellular signaling cascade activating Pdx-1 (16-20).

For a transcription factor required for pancreatic development (6), and whose conditional deletion in mice leads to diabetes (8,21), surprisingly little is known about Pdx-1 target genes. In beta cells, Pdx-1 is reported to directly control insulin gene expression (2) as well as the expression of the genes encoding glucose transporter 2 (GLUT2/SLC2a2) (22), islet amyloid polypeptide precursor (IAPP) (23), Pax4 (24), synaptotagmin 1 (25), and Pdx-1 itself (26,27). Like many other homeodomain factors, Pdx-1 also represses expression of some target genes. One such example is glucagon. Normally expressed in pancreatic alpha cells, glucagon may be under direct repressional control by Pdx-1 in beta cells (8,28). However, the number of target genes is certainly much greater, as Pdx-1+/− mice display pleiotropic phenotypes (5,7).

With the aid of genomic sequencing and associated technologies, it is now known that the number of transcripts (29-31) and genetic regulatory domains (32,33) is far greater than previous estimates. To identify new Pdx-1 binding sites, including sites in novel regulatory regions, we have used chromatin immunoprecipitation (ChIP) (34) and a new promoter microarray produced by the Beta Cell Biology Consortium (Mouse PromoterChip BCBC-5B). In addition to promoter elements, this array contains enhancers and highly conserved regions that may contain novel regulatory elements or transcripts. Additionally, genomic elements are included that represent microRNAs, an abundant class of regulatory RNAs that also have implications for gene regulation. Lastly, the microarray contains putative regulatory regions identified through Serial Analysis of Chromatin Occupancy (SACO) (35), a technique that combines ChIP with a modification of LongSAGE (Serial Analysis of Gene Expression) (36). Review of the target gene list indicates that Pdx-1 binds to regulatory elements in genes involved in virtually every aspect of the insulin secretory process in beta cells, from glucose uptake and metabolism to insulin processing and release. The data provide a comprehensive framework for understanding Pdx-1 knock-out mouse models that display beta cell dysfunction and systemic glucose intolerance (5,7,8,21).

It is highly likely that Pdx-1 binding sites are embedded within larger genetic regulatory domains containing multiple transcription factor binding sites. To test this idea, we made a second SACO library from NIT-1 cells using antibodies directed against NeuroD1/BETA2. NeuroD1 is a critical determinant of pancreatic endocrine cell differentiation (37), and both Pdx-1 and NeuroD1 regulate insulin expression (2,38). The results from this parallel screen reveal a high degree of target overlap between these two factors and suggests that the identified binding sites may denote regulatory modules (39,40) that are occupied by multiple factors controlling gene expression in a combinatorial fashion. One co-regulated target that we identified is the microRNA, miR-375, believed to negatively regulate insulin secretion (41). This result uncovers a previously unknown genetic regulatory pathway involving microRNA in pancreatic beta cells and provides a model wherein Pdx-1 and NeuroD1 control both insulin gene expression and its secretion via miR-375.

**EXPERIMENTAL PROCEDURES**

**Cell culture** - Mouse insulinoma NIT-1 cells were grown in Kaighn’s modification of Ham’s F12 medium (F12K) (ATCC, Manassas, VA) which contains 1.5 g/L sodium bicarbonate, supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Hyclone, Logan, UT), 100 units/ml penicillin, and 100 units/ml streptomycin (Invitrogen, Carlsbad, CA). The inducible DN-Pdx-1 rat insulinoma INS-1 cell line (lacking the first 79 amino acids) was produced by C.B. Wollheim and H. Wang (28). Stable and control INS-1 cells were both grown in RPMI medium (Invitrogen) supplemented with 10 mM Hepes pH 7.4, 1 mM sodium pyruvate, 50 μM 2-mercaptoethanol, 10% FBS, penicillin, and streptomycin. Cells were grown under selection with 50 μg/ml G418 sulfate (Promega, Madison, WI) and 50 μg/ml hygromycin (Invitrogen) and were induced by 300 ng/ml doxycycline (Dox) (Sigma, St. Louis, MO) for 4 d, with media and drugs changed after 2 d. All cells were grown at 37°C in a humidified chamber in 5% CO2.
Chromatin immunoprecipitation (ChIP) - Media was removed from confluent 10 cm plates of NIT-1 cells (~5x10^6 cells per plate) and replaced with 1% paraformaldehyde in 1xPBS for 15 min at room temperature (RT). The plates were washed twice with 1xPBS on ice and harvested in ice-cold buffer consisting of 100 mM Tris-HCl (pH 9.4) and 10 mM dithiothreitol (DTT). Cells were centrifuged at 1000 x g at 4°C for 5 min followed by a 1xPBS wash. Cell pellets were lysed in 600 μl buffer containing 20 mM Tris-HCl pH 8.1, 150 mM NaCl, 0.1% SDS, 0.5% Triton X-100, and 1x protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN) and then sonicated to an average size of 2kb using a bath sonicator (5 x 30 sec, 140 W pulses with 30 sec rest intervals in ice water) (Misonix, Inc, Farmingdale, NY). Samples were centrifuged and supernatants (400 μg protein) were pre-cleared for 1 h at 4°C with protein A-sepharose (Invitrogen) that was previously blocked with bovine serum albumin (0.25%) and glycogen (0.24 mg/ml). Samples were centrifuged and supernatants were incubated overnight at 4°C with 0.75 μl (~25 μg) rabbit anti-Pdx-1 antiserum raised against the N-terminus (5), 1ul of C-terminal anti-Pdx-1 antiserum (Chemicon International, Temecula, CA), or 1 μg purified rabbit anti-NeuroD1 raised against amino acids 122-165 (42). An equivalent amount of control rabbit antiserum (Sigma) was added to IgG samples and incubated overnight. Anti-Pdx-1 and NeuroD1 complexes were captured with 50 μl protein A-sepharose (50% slurry) for 2 h at 4°C, then sequentially washed with lysis buffer 4 times for 10 min each, LiCl buffer (10 mM Tris-HCl pH 8.1, 250 mM LiCl, 1 mM EDTA, 1% NP-40, 1% sodium deoxycholate) once for 10 min, and TE buffer (50 mM Tris-HCl pH 8.1, 1 mM EDTA) once for 30 min and again for 5 min. Elution buffer (100 mM NaHCO₃ and 1% SDS) was added directly to the beads and the immuno-complexes were dissociated in two sequential washes of 200 μl each for 15 min at RT. The bead supernatants were pooled and incubated at 65°C overnight to reverse the formaldehyde crosslinking. The samples were extracted with 25:24:1 phenol:chloroform:isoamyl alcohol (Invitrogen) and nucleic acids were precipitated with ethanol. Each ChIP sample was resuspended in 100 μl 10 mM Tris-HCl pH 8.0.

Mouse pancreatic islet preparation – Pancreata from eight C57BL mice aged 4 to 8 mo were injected with 1 ml cold Hanks Balanced Salt Solution (HBSS, Invitrogen) containing 0.3 mg/ml Liberase R1 (Roche Applied Science). Pancreata were then dissected and minced before incubating in a total volume of 5 ml HBSS at 37°C for 25 min to allow digestion to occur. Digestions were stopped with the addition of 45 ml HBSS containing 10 % FBS. Tissue suspensions were centrifuged at 1000 rpm for 1 min, supernatants removed, and the pellets were resuspended in 25 ml HBSS + 10 % FBS. Pancreatic islets were visualized by addition of 0.5 mg/ml diphenylthiocarbazone (Sigma) and were placed individually into fresh HBSS and then into RPMI media containing 5 mM glucose, 10 % FBS, and pen/strep supplements. Approximately 400 islets were maintained overnight at 37°C in a humidified chamber in 5% CO₂ before performing the ChIP assays.

Reverse transcription - INS-1 control and DN-Pdx-1 expressing cells (5x10⁵) were plated onto 12-well plates and treated with or without 300 ng/ml doxycycline for 4 d. Total RNA was isolated by extraction with Trizol reagent (Invitrogen), and 500 ng was used for first-strand cDNA synthesis in a reaction containing 50 ng random primers, 500 μM dNTPs, 1x first-strand buffer, 10 mM DTT, 200 units M-MLV reverse transcriptase (Invitrogen), and 20 units RNasin ribonuclease inhibitor (Promega) in 20 μl reaction volume. Samples were diluted 1:5 before using in quantitative PCR reaction.

Quantitative PCR - ChIP DNA or cDNA (2 μl) was analyzed in a 10 μl real-time PCR reaction (qPCR) containing 1x PCR buffer, 2.5 mM MgCl₂, 200 μM dNTPs, 1x SYBR green, 0.1 unit Platinum Taq DNA polymerase (all PCR reagents from Invitrogen), and 1 μM primers (IDT, Coralville, IA). Primers were designed using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) (43) using default parameters excepting: rodent and simple repeat mispriming library was used; product size was selected as 50-150 bp; optimal primer size was 23 nt; primer Tm was between the ranges of 65 ° and 70°C, with an optimal Tm of 68°C; max self complementarity was 4.00; and max poly-x was 3. For ChIP confirmations, input sequences for
Primer3 analysis were 400 bp regions surrounding the mean of the array or SACO loci. In a few cases, additional primers were designed outside this 400 bp window. PCR reactions were performed using a PTC-200 DNA engine cycler with a CFD-3200 Opticon detection system (MJ Research, Inc, Waltham, MA) for one cycle at 95°C (1 min) and 45 cycles of 94°C (15 sec) and 68°C (40 sec). Standard curves for each primer set were serial dilutions of ChIP input DNA that had been processed in a similar manner to ChIP DNA. Quantitative ChIP data was derived from the standard curve, and was expressed as “fold change over average control”, in which the sample value was divided by the average of six control values, multiplied by 100. Pdx-1 positive signals were considered to be 2-fold above the average control value, and 2-fold above the average IgG ChIP signal. For RT-PCR, “percent change” was calculated from samples treated with or without Dox. PCR products were further analyzed on 4% NuSieve GTG low-melt agarose gels (Cambrex Bio Science, Inc, Rockland, ME). Only single-band PCR products were considered for analysis. Primer sequences are listed in Supplemental Table S1.

**Construction of promoter microarray** – The Mouse PromoterChip BCBC 5B ([http://www.cbil.upenn.edu/EPConDB/](http://www.cbil.upenn.edu/EPConDB/)) contains over 18,000 1kb tiles amplified from mouse genomic DNA, and includes promoters, enhancers, conserved sequences, microRNAs, and Pdx-1 SACO elements. The array contains 5100 proximal promoters, as determined from full-length cDNA libraries and Reference Sequences (RefSeqs), and 7728 enhancers, defined as a 1kb sequence containing the most putative transcription factor binding sites (based upon a combined TRANSFAC hit (p-value) and human-mouse conservation score) within 5kb of the transcriptional start site (TSS). The array also includes putative regulatory sequences highly conserved between human and pufferfish (1172 tiles) (44) and pancreatic-specific genes conserved between human and chicken (318 tiles) (45). 615 of the tiles span microRNA and surrounding sequences. Lastly, 1981 tiles were added that are potential regulatory regions as identified through the Pdx-1 SACO assay.

**ChIP-on-chip assay** - Pdx-1 and IgG ChIP DNA from two independent experiments, each performed in duplicate, were amplified by ligation-mediated PCR (LM-PCR) and fluorescently labeled as described (46). Labeled DNA was hybridized to the Mouse PromoterChip BCBC-5B.

**SACO assay** - The Pdx-1 SACO library was made as described (35). Briefly, sonicated ChIP DNA was first amplified by LM-PCR, then digested with NlaIII (New England Biolabs, Beverly, MA) leaving an overhanging CATG. Adapters with corresponding CATG overhangs that contained a recognition site for MmeI (NEB) were then ligated. MmeI cleaves 20-22 bp downstream from its binding site, leaving an NN overhang. The digested fragments were then self-ligated to form “ditags”. The Mme-adapters were cleaved using NlaIII, concatamerized, and subcloned into pZERO-2 (Invitrogen). Sequencing of concatamers was performed by Rexagen Corporation (Seattle, WA).

**Statistical Analysis** - Detailed descriptions of the procedures are provided in the MIAME supplement. In brief, after examination of QA/QC metrics and normalization, a regularized t-test was used to determine enrichment using a level of significance of 0.05. Putative targets were identified as those elements meeting both the statistical criteria as well as a biological threshold (i.e., fold > 1.4). For all tables, q-values are reported (47). The q-value of a test measures the proportion of false positives incurred (the false discovery rate) when that particular test is called significant. Annotation of the array was done using the Mouse (mm8) assembly. Elements were mapped to target genes using the Entrez Gene Identifier. Categorical data analysis was performed to examine if particular TRANSFAC motifs, Gene Ontology categories or KEGG pathways were over-represented in the candidate target gene list compared to the expected frequency of those categories based on the entire array.

**RESULTS**

**Quality of Pdx-1 ChIP** - To test the quality of the Pdx-1 ChIP, we measured the signal obtained on the well-characterized Pdx-1 target gene, insulin. Pdx-1 ChIPs were performed using NIT-1 insulinoma cells, a mouse beta cell line, and the results were analyzed using quantitative real time PCR (qPCR). Fig. 1 demonstrates the specificity of the Pdx-1 antiserum, as there was an
approximately 14-fold enrichment for the insulin promoter using anti-Pdx-1 versus IgG antiserum. As an additional control, genes not expressed in this cell line were also examined, demonstrating that the Pdx-1 antibody does not precipitate non-specific sequences.

**Analysis of Pdx-1 genomic binding sites** – A collection of high confidence binding sites was identified by hybridizing the Pdx-1 ChIP DNA and control IgG DNA to the Mouse PromoterChip BCBC-5B microarray. A high confidence binding site was defined as having a fold change over IgG greater than 1.4 and p-value <0.05, as well as meeting the QA/QC metrics for the array (based on PCR flags). 817 elements on the array were identified as high confidence sites (Supplemental Table S2). This included 298 Mouse-Human conserved elements, 230 elements from the Pdx-1 SACO library, 152 proximal promoter elements, 107 Human-Pufferfish conserved elements, 22 Human-Chicken conserved elements and 8 elements that were within 5kb of microRNAs (Supplemental Table S2). We analyzed the Pdx-1 positive elements for occurrences of a Pdx-1 binding motif as identified by the TRANSFAC database (48). We found that this motif was highly enriched (χ² = 47.05, df = 1, P < 1 X 10⁻⁵), when compared with all array tiles (subtracting out the SACO elements because they are already considered to be enriched for Pdx-1 motifs), providing additional evidence that the sites identified are valid Pdx-1 target sites.

To begin to assign functional significance to the Pdx-1 binding sites, we mapped the elements to 583 gene targets (based on unique Entrez Gene ID) after duplicate genes were removed. To confirm whether these high confidence binding sites were authentic, we randomly selected 30 for repeat ChIP assays using specific primers. 100% of the sites confirmed by repeat ChIP assay and qPCR analysis (Fig. 2A). As qPCR analysis is more sensitive than the hybridization signal obtained from microarray analysis, in this case a positive signal was considered to have a fold change (FC) of 2.0 above a non-specific IgG ChIP and a FC of 2.0 above a selection of negative control genes. These negative control genes were selected because they were transcriptionally silent in NIT-1 cells, and the data are displayed at the far right in Fig. 2A. As an additional control, we repeated the ChIP assays using Pdx-1 antiserum that recognizes another epitope and obtained the same confirmation rate (data not shown). Furthermore, we performed ChIP assays for Pdx-1 and IgG using isolated mouse pancreatic islets and confirmed the majority (7/10) of targets tested (Fig. 2B).

**Pdx-1 target genes control critical functions of beta cell biology** - To categorize the properties of the genes associated with Pdx-1 binding sites, we utilized the Gene Ontology (GO) biological process classification (http://geneontology.org) (Table 1 and Supplemental Table S3). Genes associated with metabolism-related GO categories were significantly over-represented in the candidate target list. These categories included primary and cellular metabolism, as well as regulation of metabolism (all p-values < 1 x 10⁻⁴). Other significantly over-represented GO categories include cell organization and biogenesis (P=0.0019), regulation of cellular physiological process (P=0.0024), cell division (P=0.0053), cell development (P=0.011) and establishment of localization (P=0.021). Members of two pathways were also significantly over-represented in the candidate gene list: maturity onset diabetes of the young (KEGG MMU04950) and n-glycan biosynthesis (KEGG MMU00510) (both p-values < 0.05).

**Pdx-1 regulates transcription of identified target genes** - To test the functionality of the newly identified Pdx-1 binding sites, we measured transcript levels of 31 protein-coding genes located within 5kb of a high confidence Pdx-1 binding site. Two categories of genes were selected based on GO classifications: transcription co-activators and mediators of intracellular trafficking and exocytosis. Genes in each category were randomly selected and analyzed by reverse-transcriptase PCR (RT-PCR) in rat insulinoma INS-1 cells. 29 of 31 genes were expressed, suggesting that the sites of Pdx-1 binding are highly correlated with transcription of the associated gene (Fig. 3).

To address whether these transcribed genes are under control of Pdx-1, we measured transcript levels in the presence or absence of a doxycycline-inducible dominant-negative Pdx-1 inhibitor (Fig. 4A, inset) (28,49). This dominant-negative inhibitor (DN-Pdx-1) lacks the N-terminal transactivation domain and thus interferes
with endogenous WT Pdx-1 activity by competing for promoter sites. Stable induction of this mutant protein in INS-1 cells reduces the expression of known Pdx-1 target genes and impairs agonist-stimulated insulin secretion (28,49). To test whether DN-Pdx-1 induction influenced the expression of high confidence Pdx-1 target genes, we performed quantitative reverse-transcriptase PCR (qRT-PCR) on control and DN-Pdx-1 INS-1 cells treated for 4 days with or without doxycycline. Of 14 target genes functioning in exocytosis and intracellular trafficking, 9 were significantly down-regulated by DN-Pdx-1 (Fig. 4A), indicating that these genes are activated by WT Pdx-1. Of 15 target genes functioning as transcription co-activators, expression changes after doxycycline treatment were variable, with several genes being significantly up-regulated as well as several that were down-regulated (Fig. 4B). These data demonstrate that the majority of genes bound by Pdx-1 are also regulated in a Pdx-1-dependent manner in this cell culture system.

Pdx-1 and NeuroD1 shared targets - Pdx-1 and NeuroD1 cooperate to regulate transcription of the insulin gene (50,51). Whether the binding sites for these two factors coexist in other genes has not been extensively examined, however. To address this question, we sequenced a NeuroD1 SACO library in parallel to the Pdx-1 SACO library. Both libraries were prepared from NIT-1 cells, allowing us to identify DNA sequences that were occupied by both factors. Pdx-1 and NeuroD1 ChIP DNA was processed into ~21 bp genomic signature tags (GSTs) as described by Impey et al (35) and these GSTs were mapped in relation to each other. Pdx-1 and NeuroD1 GSTs that fell within 500 bp of each other confirmed at a high rate (~90%) in repeat ChIP assays (Fig. 5). We next located target genes that were within 5kb of either GST in our high confidence data set. In total, 440 RefSeq genes and microRNAs were found that had binding sites for both factors (Table 2 and Supplemental Table S4).

Interestingly, one gene targeted by both factors is the microRNA, miR-375. Two distinct NeuroD1 binding domains were identified, the first located 500 bp upstream from the microRNA 5’ end, and the second located 1700 bp downstream (Fig. 6). We considered the first domain to be the proximal promoter, while the second domain a distal enhancer. Two potential NeuroD1 binding sites (E-boxes) are located in the proximal promoter region spanned by the ChIP primers. Likewise, the distal enhancer region also contains two conserved E-boxes that are spanned by the downstream ChIP primers (Fig. 6). Pdx-1 binding was also detected at both the proximal promoter and the distal enhancer (Fig. 6), however, no canonical Pdx-1 binding motifs were present at either site, raising the possibility that binding was through a non-canonical site or through an indirect mechanism.

DISCUSSION

Characterization of the family of Pdx-1 and NeuroD1 genomic targets has many implications for the understanding and treatment of diabetes. Some of these genes may be potential drug targets. Moreover, the ability to convert ES and liver cells into the pancreatic lineage may be facilitated by knowledge of the transcriptional and signaling networks in beta cells (52-55). Finally, this analysis may identify new genetic markers for use in isolating pancreatic progenitors and mature beta cells (56). With these goals in mind, we have identified 583 new Pdx-1 candidate target genes in NIT-1 cells using a combined approach of hybridization and sequence-based technologies. We found that 29 of 31 Pdx-1 target genes tested had detectable transcript levels in INS-1 cells (Fig. 3). Expression of the majority was altered by induction of a dominant-negative version of Pdx-1 (Fig. 4). Furthermore, we have identified 440 genomic loci that contain linked Pdx-1 and NeuroD1 binding sites by aligning genomic signature tags (GSTs) from two SACO libraries in parallel. This analysis suggests that a significant fraction of Pdx-1 and NeuroD1 binding sites exist within regulatory modules, where they may control gene expression in a synergistic manner.

The list of transcriptional targets presented here is intended to aid in the understanding of Pdx-1 and NeuroD1 biology. Results from Brissova et al (5) using isolated islets from Pdx-1<sup>+/−</sup> mice point to impaired beta cell metabolism and Ca<sup>2+</sup> signaling, while Johnson et al (7) demonstrated defects in islet architecture and increased islet cell apoptosis. The Pdx-1 targets presented here may help explain the basis for these phenotypes. For example, GO classification indicated that metabolism-related genes were significantly over-represented, providing further
evidence that Pdx-1 is critical for maintaining the proper metabolic state of beta cells (57). Our data also implicate Pdx-1 in regulating genes involved in Ca\textsuperscript{2+} signaling, for example, protein kinase C isoforms (PKC alpha and epsilon) and voltage-dependent calcium channels (Ca\textsubscript{1.2}, 1.4, and 3.2). Additionally, several target genes are involved in synthesis of inositol 1,4,5-triphosphate (IP\textsubscript{3}), a second messenger critical for opening intracellular Ca\textsuperscript{2+} channels. These include phosphatidylinositol (PI) 4-kinases (PI4KB and PI4K2B) and phospholipase C beta. Still other Pdx-1 target genes may explain, in part, the increased apoptosis seen in Pdx-1\textsuperscript{+/-} islets (7). Of note, one target is the transcription factor p53, a master regulator of cell growth arrest and cell death (58). Also, several target genes are involved in caspase signaling, including IL-1 receptor, Fas-associated death domain, and the effector caspase, Casp3 (59). Misregulation of these genes may sensitize the cell to apoptotic stimuli. Identification of these Pdx-1 targets provides a genetic framework for understanding the Pdx-1 deficient mouse models.

Pdx-1 expression has been tested for its ability to convert ES cells (10) and liver cells (11-15) into beta cells. Data from our study and others (52-55) suggest that to re-enforce commitment to a particular lineage, multiple transcription factors may be required. For example, we have found that the homeobox protein Pax6 is a target of Pdx-1 (Table 1) and, in turn, Pax6 has been reported to regulate Pdx-1 (60). This relationship defines a multicomponent feedback loop that potentially can provide long-term stability to the system (54,61-63).

As another example, we have identified a potential feed-forward circuit between Pdx-1 and NeuroD1. This type of network may impart sensitivity to sustained, as opposed to transient, inputs (54,61-63). We discovered that Pdx-1 binds to the NeuroD1 promoter (Table 1), and in turn show that both Pdx-1 and NeuroD1 may cooperate to drive downstream expression of 440 additional genes as identified by our SACO analyses (Supplemental Table S4). In support of this hypothesis, we identified several potential Pdx-1 binding sites 3.5 kb upstream from the NeuroD1 transcriptional start site that are conserved between humans and fish.

One of the downstream target genes of both NeuroD1 and Pdx-1 is the microRNA, miR-375. This microRNA has been shown to negatively regulate insulin secretion in mouse insulinoma MIN6 cells (41) but to date no studies have investigated the regulation of microRNAs in pancreatic beta cells. We show here that there are at least two regions of the miR-375 locus, termed the proximal promoter and distal enhancer, which are bound by both NeuroD1 and Pdx-1 (Fig. 6). As these two transcription factors are critical for beta cell development (64), we anticipate that miR-375 will also play a role in beta cell development and may be controlled temporally during embryogenesis by NeuroD1 and Pdx-1. Future work will focus on the expression patterns of these three regulators during beta cell differentiation.

REFERENCES
1. Bonner-Weir, S., and Weir, G. C. (2005) Nat Biotechnol 23(7), 857-861
2. Ohlsson, H., Karlsson, K., and Edlund, T. (1993) EMBO J 12(11), 4251-4259
3. Leonard, J., Peers, B., Johnson, T., Ferreri, K., Lee, S., and Montminy, M. R. (1993) Mol Endocrinol 7(10), 1275-1283
4. Miller, C. P., McGehee, R. E., Jr., and Habener, J. F. (1994) EMBO J 13(5), 1145-1156
5. Brissova, M., Shiota, M., Nicholson, W. E., Gannon, M., Knobel, S. M., Piston, D. W., Wright, C. V., and Powers, A. C. (2002) J Biol Chem 277(13), 11225-11232
6. Jonsson, J., Carlsson, L., Edlund, T., and Edlund, H. (1994) Nature 371(6498), 606-609
7. Johnson, J. D., Ahmed, N. T., Luciani, D. S., Han, Z., Tran, H., Fujita, J., Misler, S., Edlund, H., and Polonsky, K. S. (2003) J Clin Invest 111(8), 1147-1160
8. Ahlgren, U., Jonsson, J., Jonsson, L., Simu, K., and Edlund, H. (1998) Genes Dev 12(12), 1763-1768
9. Fujitani, Y., Fujitani, S., Boyer, D. F., Gannon, M., Kawaguchi, Y., Ray, M., Shiota, M., Stein, R. W., Magnuson, M. A., and Wright, C. V. (2006) Genes Dev 20(2), 253-266
10. Miyazaki, S., Yamato, E., and Miyazaki, J. (2004) Diabetes 53(4), 1030-1037
11. Ferber, S., Halkin, A., Cohen, H., Ber, I., Einav, Y., Goldberg, I., Barshack, I., Seijffers, R., Kopolovic, J., Kaiser, N., and Karasik, A. (2000) Nat Med 6(5), 568-572
12. Sapir, T., Shternhall, K., Meivar-Levy, I., Blumenfeld, T., Cohen, H., Skutelsky, E., Eventov-Friedman, S., Barshack, I., Goldberg, I., Pri-Chen, S., Ben-Dor, L., Polak-Charcon, S., Karasik, A., Shimon, I., Mor, E., and Ferber, S. (2005) Proc Natl Acad Sci USA 102(22), 7964-7969
13. Miyatsuka, T., Kaneto, H., Kajimoto, Y., Hirota, S., Arakawa, Y., Fujitani, Y., Umayahara, Y., Watada, H., Yamasaki, Y., Magnuson, M. A., Miyazaki, J., and Hori, M. (2003) Biochem Biophys Res Commun 310(3), 1017-1025
14. Li, W. C., Horb, M. E., Tosh, D., and Slack, J. M. (2005) Mech Dev 122(6), 835-847
15. Zalzman, M., Gupta, S., Giri, R. K., Berkovich, I., Sappal, B. S., Karnieli, O., Zern, M. A., Fleischer, N., and Efrat, S. (2003) Proc Natl Acad Sci U S A 100(12), 7253-7258
16. Buteau, J., Roduit, R., Susini, S., and Prentki, M. (1999) Diabetologia 42(7), 1327-1334
17. Li, Y., Cao, X., Li, L. X., Brubaker, P. L., Edlund, H., and Drucker, D. J. (2005) Diabetes 54(2), 482-491
18. Stoffers, D. A., Kieffer, T. J., Hussain, M. A., Drucker, D. J., Bonner-Weir, S., Habener, J. F., and Egan, J. M. (2000) Diabetes 49(5), 741-748
19. Wang, X., Cahill, C. M., Pineyro, M. A., Zhou, J., Doyle, M. E., and Egan, J. M. (1999) Endocrinology 140(10), 4904-4907
20. Stoffers, D. A., Kieffer, T. J., Hussain, M. A., Drucker, D. J., Bonner-Weir, S., Habener, J. F., and Egan, J. M. (2000) Diabetes 49(5), 741-748
21. Wang, X., Cahill, C. M., Pineyro, M. A., Zhou, J., Doyle, M. E., and Egan, J. M. (2005) Diabetes 54(2), 482-491
22. Gerrish, K., Cissell, M. A., and Stein, R. (2001) J Biol Chem 276(51), 47775-47784
23. Marshak, S., Benshushan, E., Shoshkes, M., Havin, L., Cerasi, E., and Melloul, D. (2000) Mol Cell Biol 20(20), 7583-7590
24. Wang, H., Maechler, P., Ritz-Laser, B., Hagenfeldt, K. A., Ishihara, H., Philippe, J., and Wollheim, C. B. (2001) J Biol Chem 276(27), 25279-25286
25. Ng, P., Wei, C. L., Sung, W. K., Chiu, K. P., Lipovich, L., Ang, C. C., Wang, C. J., Vogelstein, B., Kinzler, K. W., and Velculescu, V. E. (2002) Nat Biotechnol 20(5), 508-512
26. Impey, S., McCorkle, S. R., Cha-Molstad, H., Dwyer, J. M., Yochum, G. S., Boss, J. M., McWeeny, S., Dunn, J. D., Mandel, G., and Goodman, R. H. (2004) Cell 119(7), 1041-1054
27. Howard, M. L., and Davidson, E. H. (2004) Dev Biol 271(1), 109-118
28. Levine, M., and Davidson, E. H. (2005) Proc Natl Acad Sci USA 102(14), 4936-4942
41. Poy, M. N., Eliasson, L., Krutzfeldt, J., Kuwajima, S., Ma, X., Macdonald, P. E., Pfeffer, S., Tuschl, T., Rajewsky, N., Rorsman, P., and Stoffel, M. (2004) *Nature* **432**(7014), 226-230
42. Poulin, G., Lebel, M., Chamberland, M., Paradis, F. W., and Drouin, J. (2000) *Mol Cell Biol* **20**(13), 4826-4837
43. Rozen, S., and Skaletsky, H. (2000) *Methods Mol Biol* **132**, 365-386
44. Woolfe, A., Goodson, M., Goode, D. K., Snell, P., McEwen, G. K., Vavouri, T., Smith, S. F., North, P., Callaway, H., Kelly, K., Walter, K., Abnizova, I., Gilks, W., Edwards, Y. J., Cooke, J. E., and Elgar, G. (2005) *PLoS Biol* **3**(1), e7
45. Schug, J., Schuller, W. P., Kappen, C., Salbaum, J. M., Bucan, M., and Stoeckert, C. J., Jr. (2005) *Genome Biol* **6**(4), R33
46. Friedman, J. R., Larris, B., Le, P. P., Peiris, T. H., Arsenlis, A., Schug, J., Tobias, J. W., Kaestner, K. H., and Greenbaum, L. E. (2004) *Proc Natl Acad Sci U S A* **101**(35), 12986-12991
47. Storey, J. D., and Tibshirani, R. (2003) *Proc Natl Acad Sci U S A* **100**(16), 9440-9445
48. Matys, V., Fricke, E., Geffers, R., Gossling, E., Haubrock, M., Hehl, R., Hornischer, K., Karas, D., Kel, A. E., Kel-Margoulis, O. V., Kloos, D. U., Land, S., Lewicki-Potapov, B., Michael, H., Munch, R., Reuter, I., Rotert, S., Saxel, H., Scheer, M., Thiele, S., and Wingender, E. (2003) *Nucleic Acids Res* **31**(1), 374-378
49. Wang, H., Iezzi, M., Theander, S., Antinozzi, P. A., Gauthier, B. R., Halban, P. A., and Wollheim, C. B. (2005) *Diabetologia* **48**(4), 720-731
50. Aramata, S., Han, S. I., Yasuda, K., and Kataoka, K. (2005) *Biochim Biophys Acta* **1730**(1), 41-46
51. Qiu, Y., Guo, M., Huang, S., and Stein, R. (2002) *Mol Cell Biol* **22**(2), 412-420
52. Jensen, J. (2004) *Dev Dyn* **229**(1), 176-200
53. Murtaugh, L. C., and Melton, D. A. (2003) *Annu Rev Cell Dev Biol* **19**, 71-89
54. Servitja, J. M., and Ferrer, J. (2004) *Diabetologia* **47**(4), 597-613
55. Wilson, M. E., Scheel, D., and German, M. S. (2003) *Mech Dev* **120**(1), 65-80
56. Keller, G. (2005) *Genes Dev* **19**(10), 1129-1155
57. Gauthier, B. R., Brun, T., Sarret, E. J., Ishihara, H., Schaad, O., Descombes, P., and Wollheim, C. B. (2004) *J Biol Chem* **279**(30), 31121-31130
58. Vogelstein, B., Lane, D., and Levine, A. J. (2000) *Nature* **408**(6810), 307-310
59. Ichijo, H. (1999) *Oncogene* **18**(45), 6087-6093
60. Samaras, S. E., Cissell, M. A., Gerrish, K., Wright, C. V., Gannon, M., and Stein, R. (2002) *Mol Cell Biol* **22**(13), 4702-4713
61. Milo, R., Shen-Orr, S., Itzkovitz, S., Kashtan, N., Chklovskii, D., and Alon, U. (2002) *Science* **298**(5594), 824-827
62. Rao, C. V., Wolf, D. M., and Arkin, A. P. (2002) *Nature* **420**(6912), 231-237
63. Odom, D. T., Zizlsperger, N., Gordon, D. B., Bell, G. W., Rinaldi, N. J., Murray, H. L., Volkert, T. L., Schreiber, J., Rolfe, P. A., Gifford, D. K., Fraenkel, E., Bell, G. I., and Young, R. A. (2004) *Science* **303**(5662), 1378-1381
64. Kemp, D. M., Thomas, M. K., and Habener, J. F. (2003) *Rev Endocr Metab Disord* **4**(1), 5-17

**FOOTNOTES**

The authors would like to thank S. Impey, G. Yochum, J. Butler, S. Peters, S. Magill, and the Goodman lab for helpful discussions. This work was supported by the BCBC consortium grants, U01-DK072477-02 and U01-DK056947, and by the Swiss National Science Foundation (grant no 32-66907.01 to CBW).

The abbreviations used are: BCBC, Beta Cell Biology Consortium; ChIP, chromatin immunoprecipitation; Dox, doxycycline; GO, gene ontology; GST, genomic signature tag; LM-PCR, ligation-
mediated PCR; Pdx-1, pancreatic and duodenal homeobox protein-1; RefSeq, reference sequence; SACO, Serial Analysis of Chromatin Occupancy; TSS, Transcription start site.

Figure Legends

Table 1. Functional assignments for abbreviated group of Pdx-1 target genes based upon gene ontology (GO). Pdx-1 genes were assigned functions based upon the GO categories of molecular function, biological process, or cellular component, and then classified into functional categories.

Table 2. Functional assignments for abbreviated group of Pdx-1 and NeuroD1 shared target genes based upon gene ontology (GO). Target genes were assigned functions based upon the GO categories of molecular function, biological process, or cellular component, and then classified into functional categories.

Fig. 1. Specific immunoprecipitation of insulin promoter chromatin by Pdx-1 antiserum. Pdx-1-DNA complexes were isolated by ChIP from mouse insulinoma NIT-1 cells. Normal rabbit antiserum was used as a non-specific antibody control. ChIP DNA was extracted, purified, and used in a quantitative real-time PCR (qPCR) assay with primers encompassing the insulin promoter or primers designed against a panel of genes not expressed in NIT-1 cells.

Fig. 2. Confirmation of high confidence Pdx-1 binding sites. A, Of the 583 candidate target genes, 30 were randomly selected for confirmation by repeat ChIP assay and qPCR analysis. DNA from three independent experiments was used in qPCR reactions, and most primer sets were designed to the mean of the 1kb sequence spotted onto the array. 100% of the tested sites confirmed, as their Pdx-1 signal was 2-fold or greater than their IgG signal and 2-fold or greater than a panel of negative genes not expressed in NIT-1 cells. The six primer sets at the right of the figure represent negative controls. B, A subset of target genes were confirmed by ChIP assay from isolated mouse pancreatic islets. Pdx-1 and IgG ChIP assays were performed on paraformaldehyde-fixed islets from 8 mice. Standard PCR reactions for the Pdx-1 and IgG ChIPs, as well as ChIP input DNA were performed on the targets indicated.

Fig. 3. Newly-identified Pdx-1 target genes are expressed in beta cells. RT-PCR was performed on randomly-selected target genes involved in intracellular trafficking and exocytosis (top panel) and transcription co-activators (bottom panel). RT-PCR reactions either with (first lane of pair) or without (second lane of pair) reverse transcriptase are shown.

Fig. 4. Pdx-1 regulates the transcription of many newly-identified targets. A, Targets involved in intracellular trafficking and exocytosis are predominantly down-regulated by DN-Pdx-1 induction. Rat insulinoma INS-1 cells expressing a stably-inducible DN-Pdx-1 construct and INS-1 control cells were treated with or without 300 ng/ml Dox for 4 days to induce expression of DN-Pdx-1 and were analyzed by quantitative reverse-transcriptase PCR (qRT-PCR). 14 random genes falling into the categories of intracellular trafficking and exocytosis were selected for this analysis. Data is from 3 independent experiments, each of which was repeated in triplicate. Data are normalized to 18S RNA and are graphed as percent change in transcript between samples treated with or without Dox. Genes that would normally be activated by Pdx-1 show down-regulation in this assay. B, Pdx-1 target genes that encode transcriptional activators are both up- and down-regulated by DN-Pdx-1 induction. As above, 15 genes in this category were randomly selected for analysis. qRT-PCR was performed on samples from 3 independent experiments as detailed above. Inset shows an anti-Pdx-1 Western blot using cell lysates from parental or DN-Pdx-1 inducing cells, in the absence or presence of 300 ng/ml Dox.

Fig. 5. Confirmation of Pdx-1 and NeuroD1 shared targets. Of the 440 candidate target genes, 37 were randomly selected for confirmation by repeat ChIP assay and qPCR analysis. DNA from three independent experiments was used in qPCR reactions. Primer sets were designed against the mean of the
Pdx-1 and NeuroD1 genomic signature tags (GSTs). 92% (34/37) of the Pdx-1 and 89% (33/37) of the NeuroD1 tested sites confirmed, as their ChIP signal was 2-fold or greater than their IgG signal and 2-fold or greater than a panel of negative control genes not expressed in NIT-1 cells. The six primer sets at the right of the figure represent the negative controls.

**Fig.6.** NeuroD1 and Pdx-1 bind to the miR-375 locus. The miR-375 locus is graphically displayed showing chromosome position, miR-375 location, and UCSC Genome Brower conservation track. NeuroD1, Pdx-1, and IgG ChIP assays were performed from NIT-1 cells and qPCR was carried out using primers against the areas indicated. The data is graphed as “fold change” and represents the fold-enrichment over a panel of six negative control genes that are not expressed in NIT-1 cells. The data was confirmed in mouse insulinoma MIN6 cells (data not shown).

**Supplemental Table S1.** List of primer sequences used in this study. Primer name includes the size of the PCR product in base pairs. Primer locations are mapped to assembly mm5 using Build 33 by NCBI and the Mouse Genome Sequencing Consortium.

**Supplemental Table S2.** Results from Mouse PromoterChip BCBC-5B array. Each element on the array has a unique Promoter ID. Entrez gene identifier number, gene symbol, and description are given. The elements are designated either ‘Proximal Promoter’ (promoter elements), ‘Mouse Human’ (enhancer elements), ‘Pufferfish Human’ (conserved elements), ‘Chicken Human’ (conserved elements), ‘miRNAs’ (microRNA elements), or ‘Pdx1’ (Pdx-1 SACO elements). The genomic locus of the tile is given and is mapped to assembly mm8 obtained from Build 36 by NCBI and the Mouse Genome Sequencing Consortium. The heading ‘Fold’ designates fold change of Pdx-1 ChIP hybridization signal above that of a matched control IgG ChIP, and q-value is a measure of false-discovery rate.

**Supplemental Table S3.** Full list of Pdx-1 target genes. Pdx-1 genes were assigned functions based upon the GO categories of molecular function, biological process, or cellular component, and then classified into functional categories.

**Supplemental Table S4.** Full list of Pdx-1 and NeuroD1 shared target genes. Target genes were assigned functions based upon the GO categories of molecular function, biological process, or cellular component, and then classified into functional categories.
### Table 1
Abbreviated Pdx-1 target gene list

| Gene name | Gene description | Gene name | Gene description |
|-----------|-----------------|-----------|-----------------|
| **I. Cell adhesion** | | | |
| Cdh24 | cadherin-like 24 | Hmgcr | HMG-Coenzyme A reductase |
| Negr1 | neuronal growth regulator 1 | Mdh1 | malate dehydrogenase 1 |
| Pak1 | P21-activated kinase 1 | Ndufb8 | NADH dehydrogenase 1, beta8 |
| Parvb | parvin, beta | Pccb | propionyl CoA carboxylase, beta |
| **II. Cell cycle** | | | |
| Anapc5 | anaphase-promoting complex 5 | Pgam1 | phosphoglycerate mutase 1 |
| Ccnb1 | cyclin B1 | Pla2g6 | phospholipase A2, group VI |
| Ccrk | cell cycle related kinase | Tnfl | thioredoxin 1 |
| Cdk8 | cyclin-dependent kinase 8 | | |
| **III. Cell growth/death** | | | |
| Bcl7b | B-cell CLL/lymphoma 7B | Il1r1 | interleukin 1 receptor, type I |
| Casp3 | caspase 3 | Impa1 | inositol (myo)-1( or 4)-monophosphatase 1 |
| Fadd | Fas-associated via death domain | Inpp5f | inositol polyphosphate-5-phosphatase F |
| St18 | suppression of tumorigenicity 18 | | |
| **IV. Cytoskeleton** | | | |
| Epb4.1 | erythrocyte protein band 4.1 | Pde4b | phosphodiesterase 4B |
| Mark2 | MAP affinity-regulating kinase 2 | Pde10a | phosphodiesterase 10A |
| Myh9 | myosin, heavy polypeptide 9 | Pik3c2g | PI3-kinase, C2 domain, gamma |
| Pfn2 | profilin 2 | Pik4cb | PI4-kinase, catalytic, beta |
| Sntb2 | syntrophin, basic 2 | Pkc13 | phospholipase C, eta 1 |
| Sntg1 | syntrophin, gamma 1 | | |
| **V. Exocytosis** | | | |
| Pclo | piccolo | Trp53 | transformation related protein 53 |
| Rph3a | rabphilin 3A | | |
| Syn1 | synapsin 1 | | |
| Syt7 | synaptotagmin VII | | |
| Syt11 | synaptotagmin XI | | |
| Syt13 | synaptotagmin XIII | | |
| **VI. Hormone processing** | | | |
| Pcsk1 | proprotein convertase 1 | Pcsk2 | proprotein convertase 2 |
| Pcsk2 | proprotein convertase 1 | | |
| **VII. Intracellular trafficking** | | | |
| Arfl4 | ADP-ribosylation factor 4-like | Ppp2r2c | protein phosphatase 2, regulatory subunit B, gamma |
| Chm | choroideremia | Psen2 | presenilin 2 |
| Ica1 | islet cell autoantigen 1 | Prkca | protein kinase C, alpha |
| Rab10 | RAB10, member RAS family | Prkce | protein kinase C, epsilon |
| Rab21 | RAB21, member RAS family | | |
| Rab3ip | RAB3A interacting protein | | |
| Tom1L2 | target of myb1-like 2 | XI. Transporter activity | |
| Trappc2 | trafficking protein particle complex 2 | Abcc8 | ATP-binding cassette, sub-family C, member 8 |
| Vamp8 | vesicle-associated membrane 8 | Atp6v0a1 | ATPase, H+ transporting, lysosomal V0 subunit A1 |
| Vapb | VAMP associated protein B and C | Cacna1c/Ca,1.2 | calcium channel, voltage-dependent, L type, alpha 1C subunit |
| **VIII. Metabolism** | | | |
| Atp5b | ATP synthase, F1 complex, beta | Cacna1h/Ca,3.2 | calcium channel, voltage-dependent, T type, alpha 1H |
| Atp5g2 | ATP synthase, F0 complex, c2 | Kcnj11/Kir6.2 | potassium inwardly rectifying channel, subfamily J, member 11 |
| Eno1 | enolase 1 | Slc2a3 | facilitated glucose transporter 3 |
| Glud1 | glutamate dehydrogenase 1 | Slc5a1 | sodium/gluco cotransporter 1 |
| Gyk | glycerol kinase | | |
| Secretion | Transporters | Transcription | microRNAs |
|-----------|--------------|---------------|-----------|
| Ap3d1     | Abcc8        | Foxo3a        | miR-191   |
| Exoc8     | Ca, 3.2      | Id3           | miR-222   |
| Syngr4    | Glut5        | Nfkb1         | miR-375   |
| Syt13     | Kcnh6        | Stat3         | miR-425   |
| SytL3     | Ryr1         | Stat5a        | miR-488   |
Figure 1

Fold change

0  2  4  6  8  10  12  14  16

insulin
MyoD
MIS
POMC
Rex1
Nanog
b2micro
Utf1

IgG
Pdx-1
Figure 2

A. 

B. 

Pdx-1 
IgG 
Input 

Rfx3  RIK311  Epb4  chr7:104  chr4:139 

Alp6ap2  chr4:877  chr1:633  Adk  chr2:115
Figure 4

A. % change in transcript

- Arfl4
- Alpova1
- Pclo
- Rab21
- Rab30
- Rab3p
- Rabgap1L
- Rhod
- Syn1
- Syt13
- Syt7
- Tom1L2
- Vamp8
- Vapb

B. % change in transcript

- Cbfa2
- Ebf3
- Hes6
- Id3
- Klf7
- Klf9
- Maml3
- Mef2d
- Mkl2
- Myt1
- Rfx2
- Rfx3
- Rfx4
- Srf
- Thr4
Characterization of pancreatic transcription factor Pdx-1 binding sites using promoter microarray and serial analysis of chromatin occupancy
David M. Keller, Shannon McWeeney, Athanasios Arsenlis, Jacques Drouin, Christopher V. E. Wright, Haiyan Wang, Claes B. Wollheim, Peter White, Klaus H. Kaestner and Richard H. Goodman

J. Biol. Chem. published online August 30, 2007

Access the most updated version of this article at doi: 10.1074/jbc.M700899200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2007/08/31/M700899200.DC1