The homeodomain factor Pdx-1 regulates an array of genes in the developing and mature pancreas, but whether regulation of each specific gene occurs by a direct mechanism (binding to promoter elements and activating basal transcriptional machinery) or an indirect mechanism (via regulation of other genes) is unknown. To determine the mechanism underlying regulation of each specific gene, we performed a kinetic analysis of insulin transcription following adenovirus-mediated delivery of a small interfering RNA specific for Pdx-1 into insulinoma cells and pancreatic islets to diminish endogenous Pdx-1 protein. insulin transcription was measured by measuring both a long pre-mRNA species by real-time reverse transcriptase-PCR. Following progressive knock-down of Pdx-1 levels, we observed coordinate decreases in pre-mRNA levels (to about 40% of normal levels at 72 h). In contrast, mature mRNA levels showed strikingly smaller and delayed declines, suggesting that the longer half-life of this species underestimates the contribution of Pdx-1 to insulin transcription. Chromatin immunoprecipitation assays revealed that the decrease in insulin transcription was associated with decreases in the occupancies of Pdx-1 and p300 at the proximal insulin promoter. Although there was no corresponding change in the recruitment of RNA polymerase II to the proximal promoter, its recruitment to the insulin coding region was significantly reduced. Our results suggest that Pdx-1 directly regulates insulin transcription through formation of a complex with transcriptional coactivators on the proximal insulin promoter. This complex leads to enhancement of elongation by the basal transcriptional machinery.

Insulin is produced almost exclusively by the β cells of the pancreatic islets of Langerhans. This restriction of insulin production derives primarily from constraints imposed at the level of transcription of the gene encoding proinsulin (the insulin gene), rather than at the level of translation of the nascent mRNA (1, 2). Studies of the rodent insulin genes indicate that ~400 base pairs (bp) of DNA 5′ of the transcriptional start site (the insulin promoter) are sufficient to confer cell type-specific expression of insulin (3–7). Multiple discrete sequence elements within the proximal promoter region contribute to both the specificity and magnitude of insulin expression, and these elements are believed to serve as binding sites for several islet transcription factors, including Pdx-1, MafA, and BET/NeuroD (see Ref. 8 for a review). In the prevailing hypothesis of insulin transcription, the association of these transcription factors with the promoter and their subsequent interaction with ubiquitously expressed factors (e.g. E47 and p300) (9, 10) leads to the recruitment of the basal transcriptional machinery to the insulin gene. This hypothesis, however, has never been rigorously tested for the endogenous insulin gene in β cells.

The Hox-like homeodomain protein Pdx-1 is perhaps the most extensively studied β cell transcription factor. Much of the evidence implicating Pdx-1 in the regulation of insulin transcription is circumstantial, and is derived from studies of interaction of Pdx-1 with insulin promoter elements in vitro reporter gene expression studies in non-β cell lines, and loss-of-function studies in animals (11–17). It is unknown from these experiments whether Pdx-1 might control insulin transcription by a direct mechanism involving interaction with upstream promoter elements or an indirect one via regulation of other genes. In this regard, Pdx-1 is known to regulate a complex genetic hierarchy, because its disruption in both mice and humans results not only in the absence of insulin production, but also in arrested development of the early pancreatic anlage (12, 14, 18). More recent studies involving functional or physical attenuation of Pdx-1 protein in late mouse development or in cell lines (through targeted gene knock-out, antisense, or dominant negative strategies) have suggested a potentiating effect of Pdx-1 on the endogenous insulin gene (16, 19–23). On the one hand, these latter studies provide evidence for the overall positive regulation of insulin transcription by Pdx-1; on the other, many of these studies employed use of...
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Dominant negative proteins (which may have squelching effects on gene expression) and/or involved long-term functional knock-down of Pdx-1 (days to weeks), where its impact on a wide range of islet genes (glut2, glucokinase, pdx-1, nkh6.1, glucagon, and others) may have indirectly influenced insulin expression. Few of these studies have quantitatively examined the effect of physical knock-down of endogenous Pdx-1 in the short term (2–4 days); those that have reveal widely conflicting results on insulin transcription, from virtually no effect (24) to near-complete ablation (23).

To address definitively the role of Pdx-1 in the regulation of insulin, we delivered small interfering RNA (siRNA) into mouse β cell lines and islets to diminish endogenous Pdx-1 protein; we subsequently measured transcription by reverse transcriptase (RT)-PCR and quantitated transcription factor occupancy at the insulin promoter by chromatin immunoprecipitation (ChIP). To assess precisely the transcription of the insulin gene by RT-PCR, we employed a unique strategy whereby we measured total mouse insulin I and II mRNA (which reflects primarily fully processed or mature mRNA) and a mouse insulin II pre-mRNA species containing intron 2 by quantitative real-time RT-PCR. We demonstrate here that intron 2-containing transcripts possess short half-lives (9–30 min) in both mouse β cell lines and islets, whereas mature mRNA transcript displays substantially extended half-life (perhaps in excess of 24 h). We show that Pdx-1 knock-down in β cell lines and islets results in a coordinate decline in insulin transcription as measured by insulin pre-mRNA, but not as measured by mature mRNA. This decline in insulin transcription is accompanied by a fall in the association of Pdx-1 and its interacting coactivator p300 with the insulin gene, and a fall in the occupancy of RNA polymerase II (Pol II) at the insulin coding region. Our results suggest a model whereby Pdx-1 directly regulates insulin transcription through formation of a complex with transcriptional coactivators on the proximal insulin promoter. This complex appears to lead to enhancement of transcriptional elongation by components of the basal transcriptional machinery.

**EXPERIMENTAL PROCEDURES**

**Antibodies**—Rabbit polyclonal antiserum against p300 (N-15), RNA polymerase II (N-20), and actin were from Santa Cruz Biotechnology. Rabbit polyclonal antiserum against acetylated histone H3 and acetylated histone H4 were from Upstate Biotechnology. Rabbit polyclonal antiserum against Pdx-1 was a gift from Dr. M. German (University of California, San Francisco).

**Cell Culture**—The mouse insulinoma cell lines βTC3 (25) and MIN6 (26) were maintained in Dulbecco’s modified Eagle’s medium as previously described (26, 27) in 6-well tissue culture plates at a density of 5 × 10⁶ cells/well. Mouse pancreatic islets were provided by the University of Virginia Diabetes Center Islet Isolation Core Facility. Islets were picked by hand from collagenase-digested 6–8-week-old CD-1 mouse pancreas (28) using a protocol approved by the Institutional Animal Care and Use Committee. Islets were cultured in RPMI medium containing 0.1 mM glucose for 4 h, then switched to fresh medium containing either 0.1 or 25 mM glucose for 30 min and subsequently treated with 50 μg/ml of actinomycin D. At the times indicated, cells were washed once with PBS and processed for RNA isolation using the RNeasy® kit (Qiagen) according to the manufacturer’s instructions. For isolation of whole cell extract, cells were washed twice in PBS and subsequently lysed directly in the plates by adding 50 μl of Laemml buffer containing 4% SDS.

1 The abbreviations used are: siRNA, small interfering RNA; Ad-siLuc, adenovirus encoding siRNA against luciferase mRNA; Ad-siPdx, adenovirus encoding siRNA against pdx-1 mRNA; ChIP, chromatin immunoprecipitation; Pol II, RNA polymerase II; RT, reverse transcriptase; PBS, phosphate-buffered saline; pu, plaque-forming unit.

**Adenoviral Vector Construction and Infection**—A recombinant adenovirus (Ad-siPdx) containing an siRNA sequence (5′-GAAAGAGGAAA-GATAAGAAA-3′) corresponding to nucleotides 706–724 of pdx-1 (GenBank™ accession number NM_022852) was recently prepared using vector EH006 as described (29). An siRNA sequence targeting the Phoxinus pyralis luciferase gene, GL2 (30), was cloned into adenovirus (Ad-siLuc) for use as controls. Resulting adenoviruses were purified using the AdenoX® purification kit (BD Biosciences). Infectious titers of virus, as determined by end point dilution assay in HEK293 cells, were on the order of 10⁹–10¹¹ pfu/ml.

For adenoviral transduction of insulinoma cell lines, βTC3 and MIN6 cells were cultured overnight at a density of 5 × 10⁶ cells/well in 6-well tissue culture plates as described above, followed by addition of recombinant adenoviruses at a multiplicity of infection of ~2500 pfu/cell. After 5 h, virus-containing medium was removed and cells were washed once with PBS and cultured in fresh medium. At the times indicated, cells were processed for isolation of total RNA or whole cell extracts, as described above. Islets were transduced with adenoviruses as described previously (29). Briefly, islets were maintained in 6-well plates at a density of 150 islets/well; adenoviruses were added at a multiplicity of infection of 1.3 × 10⁶ pfu/islet in a total of 0.8 ml of serum-free medium. After 24 h, 2 ml of fresh medium containing serum was added and the islets were incubated for an additional 48 h, after which they were processed for total RNA isolation and whole cell extracts. Real-time RT-PCR—Five micrograms of total RNA from β cell lines and islets were reverse transcribed at 37 °C for 1 h using 15 μg of random hexamers, 0.5 mM dNTPs, 5× first strand buffer, 0.01 μg di-thiotreitol, and 200 units of Moloney murine leukemia virus reverse transcriptase (Invitrogen) in a final reaction volume of 20 μl. Real-time RT-PCR was performed as described previously (27, 31, 32), but using forward and reverse primers to amplify mature insulin mRNA (5′-GGGAGGCTGTTCTCTTTCA-3′ and 5′-GGGGAAATCTGGTGTCGA-3′), insulin pre-mRNA (5′-GGGAGGAGGTGCCTTCTCTTCA-3′ and 5′-GGGGAAATCTGGTGTCGA-3′), or β-actin (5′-AGGTGCTACACTAT-TGGCAAGCA-3′ and 5′-ACACTTCTATGGAAATTTGAG-3′). PCR were cycled 50 times using the following conditions: 95 °C for 15 s, 64 °C for 1 min. Homogeneity of products from each reaction was confirmed by melt curve analysis (as shown in Fig. 1C). Fdx-1 mRNA levels in reverse-transcribed RNA were determined using the Assay on Demand®-RT-PCR kit (product number Mn00435565_m1, Applied Biosystems) according to the manufacturer’s instructions.

Amplified products from initial PCR were subcloned into the T/A cloning vector pCR2.1 (Invitrogen), and 3–4 clones were sequenced to confirm the identity of the amplified product. The threshold cycle (Ct) methodology (27, 31) was used to calculate relative quantities of mRNA products from each sample; all samples were corrected for total input RNA by normalizing Ct values to the Ct value of β-actin message. These corrections were verified by quantitation of total RNA in each sample by fluorescence using RiboGreen® dye (Molecular Probes).

Primers for mature mRNA and pre-mRNA displayed equal efficiency for quantification of target DNA as determined in the following manner: total genomic DNA from βTC3 cells was used as a template for PCR using each primer set. Because the mature mRNA primers recognize both mouse insulin I and insulin II (total = 4 copies in genomic DNA), whereas pre-mRNA primers recognize only mouse insulin II (total = 2 copies in genomic DNA), it would be expected that Ct values would occur 1 cycle sooner for mature mRNA primers than for pre-mRNA primers (assuming both primer sets amplified with equal efficiency). We observed amplification of mature mRNA with a Ct of ~17, and amplification of pre-mRNA with a Ct of ~18. This difference of 1 cycle was reproducible across a range of dilutions of genomic DNA.

**Mathematical Modeling of RNA Decay**—RNA decay curves shown in Fig. 2 were optimized (by a modified Gauss-Newton nonlinear least squares algorithm) (33) to an exponential decay function of the form,

\[
y(t) = \exp\left(-\frac{t}{\tau}\right) + c
\]  

(1)

where y(t) is the observed time series data; t is time; α is the magnitude of exponential decay; τ is the lifetime of the exponential decay process; and c is an additive, offset constant.

**Quantitative ChIP—ChIP assays**, including quantitation of co-immunoprecipitated DNA fragments by real-time PCR, were performed as

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described previously (27, 31, 32) with the following modifications. βTC3 cells and islets were plated in 6-well dishes at 5 x 10^5 cells/well or 150 islets/well, respectively, for infection with adenovirus (as described above). Following incubation with adenovirus for 72 h, each well was washed with PBS, cross-linked for 15 min in 1% formaldehyde (in PBS), then all wells were pooled. Pooled samples were sonicated as described (27) in 600 μl of sonication buffer to yield chromatin fragments of 500–800 bp in length. Approximately 150 μl of sonicated chromatin was diluted to 1 ml in ChIP buffer (27) and subject to immunoprecipitation with the antibodies indicated in Fig. 6. 15 μl of sonicated sample was saved for quantitation of input chromatin. ChIP assays were performed on at least 3 independent occasions; for each ChIP assay, promoter samples were quantitated in triplicate as previously described (27, 31) using SYBR Green I-based real-time PCR. Data in Fig. 6 are presented as percent of input DNA recovered. Forward and reverse primer sequences, respectively, used for PCR were: proximal insulin promoter: 5'-TACCTTGCTGAGTTCTGC-3' and 5' -GCATTTTCACATCATTCCC-3'; insulin coding sequence: 5'-TGGCTTCTTACTACCCAAG-3' and 5'-ACAATGCCACGGCTTCTGC-3'.

Immunoblot Analysis—Whole cell extracts from cell lines and islets were prepared as described above. Aliquots of 5 μg of protein were resolved by electrophoresis on a 15% SDS-polyacrylamide gel followed by immunoblot analysis using anti-Pdx-1 or anti-actin antibodies (both at 1:5000 dilution). Western blots were visualized using the ECL-Plus®
RESULTS

Quantitation of Mature insulin mRNA and insulin Pre-mRNA and Determination of Their Biologic Half-lives in β Cells—In most mammals (including the human), the gene encoding preproinsulin (insulin) exists as a single gene containing two introns. However, in rats and mice the gene has undergone a duplication event during evolution, resulting in two homologous non-allelic genes (insulin I and insulin II) (1, 2). Similar to other mammals, the rodent insulin II gene contains two introns (see Fig. 1A), whereas the insulin I gene lacks the second intron. Prior studies have suggested very long half-lives (up to perhaps 80 h) of many spliced and unspliced forms of insulin mRNAs in mouse islets and β cell lines (34–38); a notable exception, however, is the insulin II intron 2-containing transcript, which appears to have a half-life on the order of minutes (37). Because both insulin genes appear to be coordinately regulated in the β cell (39, 40), we hypothesized that measurement of a short half-life insulin II transcript species would more accurately reflect immediate changes (particularly decreases) in overall transcriptional rates that might occur in our studies of insulin gene regulation.

To measure insulin mRNA species, we designed two primer sets (shown in Fig. 1A) for quantitative real-time RT-PCR. The primer set Tf/Tr was designed to amplify a 130-bp sequence in exon 2 of the mouse insulin I and II genes, and therefore targets both spliced and unspliced mRNA (total mRNA). Because unspliced insulin mRNA represents a very small fraction of total insulin mRNA (37), this primer set can be viewed as

| Table I | Real-time RT-PCR threshold cycles (Ct) for mouse mature insulin mRNA and insulin II pre-mRNA in β cell lines and islets |
|-----------------|-------------------------------------------------|---------------------------|-------------------------------|
| **Insulin II pre-mRNA** | **Mature insulin mRNA** | **Relative abundance** |
| **RT** | **–RT** | **+RT** | **–RT** | **(pre-mRNA:mature mRNA)** |
| βTC3 cells | 17.5 ± 0.1 | >35 | 12.3 ± 0.6 | >40 | 0.027 |
| MIN6 cells | 20.3 ± 0.8 | >35 | 13.9 ± 1.4 | >40 | 0.012 |
| Islets | 28.4 ± 2.6 | >35 | 14.2 ± 2.7 | >40 | 5.3 × 10⁻⁵ |

2. Relative abundance was calculated using the following formula: \(2^{(\Delta \text{Ct}(\text{pre-mRNA}) - \Delta \text{Ct}(\text{mature mRNA}))}\). As noted under "Experimental Procedures," RT-PCR primers for insulin mRNA and pre-mRNA were equally efficient.

Fig. 2. insulin transcript half-lives. βTC3 cells, MIN6 cells, and islets in either 0.1 or 25 mM glucose were incubated with actinomycin D for the times indicated, then total RNA from the cells was isolated, reverse-transcribed, and subject to quantitation for either mature insulin mRNA (panel A) or insulin pre-mRNA (panel B) by real-time PCR. To correct for variations in input RNA, all data were normalized to the quantity of β-actin message (also determined by real-time PCR). The half-lives (t₁/₂) indicated in panel B were determined by fitting the data to an exponential decay model, as detailed under "Experimental Procedures." Experiments were performed on a minimum of three independent occasions. The curves in panel B represent the best-fit model.

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representing primarily mature (or fully spliced) mRNA. The primer set Pf/Pr was designed to amplify an 82-bp fragment spanning the exon 2/intron 2 boundary, thereby targeting only a specific unspliced mRNA (pre-mRNA) of insulin II containing intron 2. Fig. 1B shows that PCR amplification of both species of mRNA from reverse-transcribed total islet RNA results in homogeneous products of expected molecular weight. Primers were subsequently used for SYBR Green I-based real-time PCR (27, 31). Fig. 1C confirms, by melt curve analysis, that homogeneous products were obtained following 50 cycles of PCR; Fig. 1D and E, shows that the real-time PCR quantitation of both insulin pre-mRNA and mature mRNA was linear over the relatively narrow range of total RNA concentrations observed in our studies, thereby demonstrating the reproducibility and linearity of our real-time assay in distinguishing small differences in target mRNA quantities. Similar linearity data were obtained for the real-time amplification of β-actin message, and for all messages amplified from reverse-transcribed RNA isolated from two mouse β cell-derived insulinoma cell lines, βTC3 and MIN6 (data not shown).

Table I shows the real-time PCR amplification threshold values for insulin mature mRNA and pre-mRNA in mouse insulinoma cell lines and islets maintained in 25 mM glucose. Interestingly, these data reveal no substantial differences in the relative amounts of mature insulin mRNA in these cells, but do show striking differences in pre-mRNA levels. Based on the calculated ratio of pre-mRNA to mature mRNA, the quantities of pre-mRNA are substantially lower than mature mRNA in all cell types. (Importantly, as noted under “Experimental Procedures,” both primer sets appeared to have identical amplification efficiencies based on a comparison of threshold values from genomic DNA.) However, although this ratio was similar for the two insulinoma cell lines, by comparison it was lower by 200–500-fold in islets. These data suggest that the cell lines produce initial transcripts in far greater quantity than islets, a finding consistent with their tumorigenic origin.

To determine the biologic half-lives of insulin mRNA species, βTC3 cells, MIN6 cells, and mouse islets were treated with actinomycin D to block transcriptional elongation, and cells were harvested at various time points for isolation and quan-
siRNA-induced Knock-down of Pdx-1 Levels in β Cell Lines Cooperatively Diminishes insulin Pre-mRNA Levels—To determine the role of Pdx-1 in insulin transcription, we knocked down endogenous Pdx-1 protein in β cell lines and subsequently analyzed cells for insulin transcription by real-time RT-PCR. To knock-down Pdx-1 levels, we treated cells with an adenovirus encoding siRNA specific for pdx-1 (Ad-siPdx). Fig. 3A shows that Ad-siPdx treatment caused a time-dependent decrease in pdx-1 mRNA levels to a nadir of ~20% of starting concentrations in both βTC3 and MIN6 cells. Conversely, untreated cells or cells treated with an adenovirus encoding siRNA specific for luciferase (Ad-siLuc) showed no change in pdx-1 mRNA levels (Fig. 3A). The fall in pdx-1 mRNA levels in Ad-siPdx-treated cells corresponded to a decrease in Pdx-1 protein in both cell types as assessed by immunoblot analysis, to levels ~5–15% of those observed in control cells (Fig. 3, B and C).

To assess the effect of Pdx-1 knock-down on insulin gene transcription in βTC3 and MIN6 β cells, we measured both insulin mature mRNA and pre-mRNA during a time course following Ad-siPdx or Ad-siLuc treatment (or no treatment). As shown in Fig. 4A, Ad-siPdx treatment resulted in no significant decline in mature insulin mRNA levels over the first 48 h of the experiment, and a decline of only 30% at 72 h relative to control cells. These results are comparable with a previous report demonstrating virtually no decline in insulin message in both cell types following antisense RNA-induced Pdx-1 knock-down (24). In striking contrast, however, pre-mRNA levels in both cell types were decreased by 40% 48 h after Ad-siPdx treatment, and by 60% 72 h after treatment. The decreases in pre-mRNA levels precisely paralleled the fall in Pdx-1 protein levels observed by immunoblot analysis (cf. Fig. 3C), highlighting the rapid effect of Pdx-1 knock-down on insulin transcription in β cell lines. Importantly, half-lives of insulin pre-mRNA and mature mRNA appear unchanged in cells treated with Ad-siPdx and Ad-siLuc, based on actinomycin D experiments performed at 72 h after viral treatment (data not shown).

siRNA-induced Knock-down of Pdx-1 in Primary Mouse Islets Diminishes insulin Pre-mRNA Levels—To determine the contribution of Pdx-1 to insulin transcription in primary cells, we treated intact mouse islets with Ad-siPdx and compared these to Ad-siLuc-treated and untreated control islets. As shown in Fig. 5A, treatment of islets with Ad-siPdx for 72 h resulted in a ~70% decrease in pdx-1 mRNA compared with control islets. This reduction in pdx-1 mRNA corresponded to ~70% reduction in Pdx-1 protein levels compared with control islets (Fig. 5B). We next measured both mature insulin mRNA and insulin pre-mRNA by real-time RT-PCR from total RNA isolated from Ad-siPdx-treated and control islets at 72 h. As shown in Fig. 5C, mature mRNA levels decreased by ~25% compared with controls, whereas pre-mRNA levels declined by
To correct for variations in input RNA, data were normalized to actin extract. Islets were harvested at 72 h for isolation of total RNA or whole cell extracts of infected islets. Data from two independent infections are shown. Data from electrophoretic mobility shift analysis (11–13, 15, 16). Data represent percent of mRNA relative to untreated cells (set at 100%). * Asterisk (*) signifies that the values are statistically different for the comparisons indicated (p < 0.05). Data represent the average of at least three independent experiments. 50%. These results are comparable with those obtained in the β cell lines, and suggest that insulin transcription in primary isolated islets is similarly regulated by Pdx-1. Taken together, the results from β cell lines and islets strongly suggest that Pdx-1 directly regulates insulin transcription derived primarily from electrophoretic mobility shift analysis in vitro and studies of reporter gene expression in mammalian cells (11–13, 15, 16). However, those and other studies did not examine the role of endogenous Pdx-1 protein upon transcription of the native, chromatin-embedded insulin gene. In this study, we employed a unique RT-PCR strategy following siRNA delivery to study transcription of the native insulin gene in the absence of Pdx-1 protein.

One complication to studying the regulation of insulin transcription by RT-PCR or Northern blot analysis is that such assays are strongly influenced by the high levels and extended half-life of the mature insulin mRNA in insulinoma cell lines and islets. It has been estimated that the β cell contains up to 40,000 insulin transcripts, exceeding even the number of β-actin transcripts (36, 37). Our results here appear to support this observation (Table I), and indicate further that the half-life of insulin mRNA is considerably in excess of 60 min, but in all likelihood is closer to 30–60 h, as reported by many other investigators (34–38). Thus, an important implication of our studies is that if insulin transcription is studied solely by methods that quantitate total mRNA, it will be difficult to interpret the effect of putative transcriptional modulators (transcription factors, glucose, cAMP, etc.), because several days may be necessary to observe changes in the rather large insulin mRNA content in β cells. In this context, an earlier report (24) that showed no effect of Pdx-1 knock-down on insulin transcription (as assessed by Northern blot analysis) in MIN6 and βTC1 cells is clarified. Indeed, our studies confirm that knock-down of Pdx-1 levels by 80–90% causes only a 30% reduction in mature insulin mRNA over 72 h, and well after clear suppression of Pdx-1 has occurred. By themselves, these data may raise doubt as to the immediate role of Pdx-1 in insulin transcription.

To overcome the potential barrier imposed by the long half-life of insulin mRNA, we examined whether a pre-mRNA species with a shorter half-life could be used to study insulin transcription. In this respect, formation of pre-mRNAs is
more closely linked to transcription, because no intervening processes such as splicing or cytoplasmic export necessarily occur. Quantitation of such short half-life pre-mRNAs have been used as a reflection of acute transcriptional regulation in other systems such as the LHβ, FSHβ, and follistatin genes, and can closely mimic results obtained from nuclear run-off assays (44, 45). We demonstrate here by real-time RT-PCR that the intron 2-containing transcripts of mouse insulin II possess remarkably short half-lives (on the order of minutes) in both βTC3 cells (A) or islets (B). C, recovery of the proximal promoter fragment following ChIP using p300 Ab and extracts from infected βTC3 cells. All data represent recovery, in percent, of each DNA fragment relative to total input DNA. Asterisk (*) signifies that the value for Ad-siPdx-treated cells is statistically different from the corresponding value for Ad-siLuc-treated cells (p < 0.05). Data represent the average of at least three independent experiments.

four important implications: 1) pre-mRNA levels reflect a downward trend in transcription much sooner (by about 24 h) than mature mRNA levels, a finding that is explained by the shorter half-life of the pre-mRNA species; 2) the close association of insulin pre-mRNA and Pdx-1 levels, in conjunction with the decrease in occupancy of Pdx-1 at the proximal insulin promoter, points to the insulin gene as an immediate downstream target of Pdx-1 (as opposed to a target downstream of a secondary or tertiary gene expression event); 3) our data suggest that a striking fraction (at least 60%) of insulin transcriptional activity is dependent upon Pdx-1 action, notwithstanding the fact that a host of cell-specific transcription factors are believed to participate at the promoter (8). We believe these findings must be considered in the context of transcription factor cooperativity at the insulin promoter. Thus, the loss of cooperative interactions between Pdx-1 and factors such as BETA2/NeuroD and E47 (15) may underlie the significant fall in transcriptional rate; and 4) our findings in the β cell lines appear to be similar to those in primary mouse islets, suggesting that the mechanism by which Pdx-1 regulates insulin transcription in insulinoma cells is likely to be very similar to that in the primary cell type.

The mechanism governing transcriptional activation by Pdx-1 has been the subject of intense investigation over the
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Promoting transcriptional elongation at the insulin gene, at least in insulinoma cells.

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