*Original Article*

# Glucose Regulation of Insulin Gene Transcription and Pre-mRNA Processing in Human Islets

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**Glucose is the primary regulator of insulin granule release from pancreatic islets. In rodent islets, the role of glucose in the acute regulation of insulin gene transcription has remained unclear, primarily because the abundance and long half-life of insulin mRNA confounds analysis of transcription by traditional methods that measure steady-state mRNA levels. To investigate the nature of glucose-regulated insulin gene transcription in human islets, we first quantitated the abundance and half-lives of insulin mRNA and pre-mRNAs after addition of actinomycin D (to stop transcription). Our results indicated that intron 1– and intron 2–containing pre-mRNAs were ~150- and 2,000-fold less abundant, respectively, than mature mRNA. 5′ intron 2–containing pre-mRNAs displayed half-lives of only ~60 min, whereas all other transcripts displayed more extended lifetimes. In response to elevated glucose, pre-mRNA species increased within 60 min, whereas increases in mature mRNA did not occur until 48 h, suggesting that measurement of mature mRNA species does not accurately reflect the acute transcriptional response of the insulin gene to glucose. The acute increase in pre-mRNA species was preceded by a sixfold increase in histone H4 acetylation and a twofold increase in RNA polymerase II recruitment at the insulin promoter. Taken together, our data suggest that pre-mRNA species may be a more reliable reflection of acute changes to human insulin gene transcriptional rates and that glucose acutely enhances insulin transcription by a mechanism that enhances chromatin accessibility and leads to recruitment of basal transcriptional machinery.**
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The β-cells of the islets of Langherhans are the only cells capable of producing and secreting physiologically relevant amounts of insulin in response to increases in extracellular blood glucose levels. In addition to its stimulation of insulin release, glucose has also been shown to stimulate the transcription of the gene encoding preproinsulin (referred to here for simplicity as the "insulin" gene) and the subsequent processing and translation of the nascent mRNA (1,2). These transcriptional and translational effects of glucose serve to maintain ongoing insulin stores after repeated glucose challenges.

The majority of data on the effect of glucose in insulin transcription derives from studies in rodent islets and cell lines. In response to glucose, members of the mitogen-activated protein kinase family, extracellular response kinase-1/2, become activated by phosphorylation and subsequently promote the nuclear translocation and/or DNA binding of the β-cell–specific transcription factors Pdx-1, MafA, and Beta2/NeuroD1, which collectively synergistically activate insulin transcription in reporter gene assays (3–5). Recruitment of histone acetyltransferases (e.g., p300) by these transactivators also leads to increases in histone acetylation at the insulin gene, thereby contributing to a more "open" and active state of insulin chromatin (6–9). In addition to its effect on gene transcription, glucose also appears to enhance the stability of insulin mRNA in rodent β-cells through a mechanism involving binding of polypyrimidine tract-binding protein to the 3′ untranslated region of the insulin mRNA (10). Thus, the cellular response to glucose results in the enhancement of both insulin transcription and insulin mRNA stability, thereby ensuring that sufficient mRNA is present to serve as substrate for insulin production.

Findings noted above in rodent islets and tumorigenic cell lines cannot necessarily be extrapolated to humans. Importantly, human islets show substantial differences in structure and organization compared with rodent islets (11); the human gene encoding preproinsulin exists only as a single allelic pair (unlike rodents, which contain two nonallelic pairs) and contains regulatory DNA elements within the promoter that are distinctly different from the rodent orthologs (12–16). A recent elegant study of insulin transcription in human islets suggested that insulin mRNA does not increase until 48 h after exposure to elevated glucose (17). Because that study measured steady-state mRNA, which is highly abundant in the β-cell, it is unclear whether the effect of glucose is on insulin transcription or mRNA stability. Thus, further studies are needed to elucidate gene transcription in human islets.

Here, we present studies describing the nature of insulin gene transcription and transcript processing in isolated human islets, particularly as they relate to changes in glucose levels. Our data suggest that pre-mRNAs (particularly intron 2–containing pre-mRNAs) may be the most reliable reflection of acute changes to human insulin gene transcription and that glucose acutely enhances transcription at the human insulin gene by opening chromatin structure and recruiting basal transcriptional machinery.

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TABLE 1
Insulin gene regions amplified and their corresponding primer sequences used for RT-PCR

| Insulin gene region amplified | Primer sequences | Amplicon size (bp) |
|---|---|---|
| Human intron 1–exon 1 (I1-E1) | F: 5′-TCAGAAGAGGCCATCAAGCA-3′<br>R: 5′-ACATGCTTCACGAGCCCAGC-3′ | 129 |
| Human intron 1 (I1) | F: 5′-ACATGCTTCACGAGCCCAGC-3′<br>R: 5′-GGTCTGTTCCAAGGGCCTTT-3′ | 109 |
| Human intron 1–exon 2 (I1-E2) | F: 5′-TGGCTGGGCTCGTGAAGCAT-3′<br>R: 5′-GCCATGGCAGAAGGACAGTG-3′ | 115 |
| Human exon 2 (E2) | F: 5′-TGTCCTTCTGCCATGGCCCT-3′<br>R: 5′-TTCACAAAGGCTGCGGCTGG-3′ | 92 |
| Human exon 2–intron 2 (E2-I2) | F: 5′-GTGAACCAACACCTGTGCGG-3′<br>R: 5′-AGGGGCAGCAATGGGCAGTT-3′ | 139 |
| Human intron 2a (I2a) | F: 5′-AGCAGGGGGTCAGGTGCACTTT-3′<br>R: 5′-TTCTGACTGGGCCACAGGGA-3′ | 85 |
| Human intron 2b (I2b) | F: 5′-CCTCACGGCAGCTCCATAGT-3′<br>R: 5′-CAGTGGGAGCCTGAACAGGT-3′ | 92 |
| Human intron 2–exon 3 (I2-E3) | F: 5′-CTCCCTGACTGTGTCCTCCTGTGTC-3′<br>R: 5′-CCAGCTCCACCTGCCCCACT-3′ | 95 |
| Human exon 3 (E3) | F: 5′-CCCTGCAGAAGCGTGGCATT-3′<br>R: 5′-CCATCTCTCTCGGTGCAGGA-3′ | 133 |
| Mouse exon 2–intron 2 (E2-I2) | F: 5′-GGGGAGCGTGGCTTCTTCTA-3′<br>R: 5′-GGGGACAGAATTCAGTGGCA-3′ | 86 |
| Mouse intron 2–exon 3 (I2-E3) | F: 5′-CCTATGTGTCTTTGCTTCTGTG-3′<br>R: 5′-AGTGCCAAGGTCTGAAGGTC-3′ | 122 |
| Mouse exon 2 (E2) | F: 5′-TGGCTTCTTCTACACACCCAAG-3′<br>R: 5′-ACAATGCCACGCTTCTGCC-3′ | 131 |

F, forward; R, reverse.

## RESEARCH DESIGN AND METHODS

Human islets were isolated from 14 cadaveric donors at facilities at Emory University, City of Hope Hospital, the University of Pennsylvania, the Joslin Diabetes Center, the University of Minnesota, or the University of Virginia. Comprehensive donor data were known for 10 of the islet preparations. Of these 10, 6 donors were male and 4 were female; their ages ranged from 36 to 59 years (average 48 years). The cold ischemia time was known for six isolations and ranged from 5 to 16 h (average 10 h). Purity of the islet preparation was available for eight isolations and ranged from 40 to 95% (average 76%). On arrival, islets were immediately placed in RPMI culture medium containing 11 mmol/l glucose, 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin (Invitrogen) and incubated overnight at 37°C and 5% $CO_2$. Mouse islets were isolated by collagenase digestion as described previously (18), using a protocol approved by the University of Virginia Animal Care and Use Committee, and then handpicked and cultured in RPMI culture medium overnight.

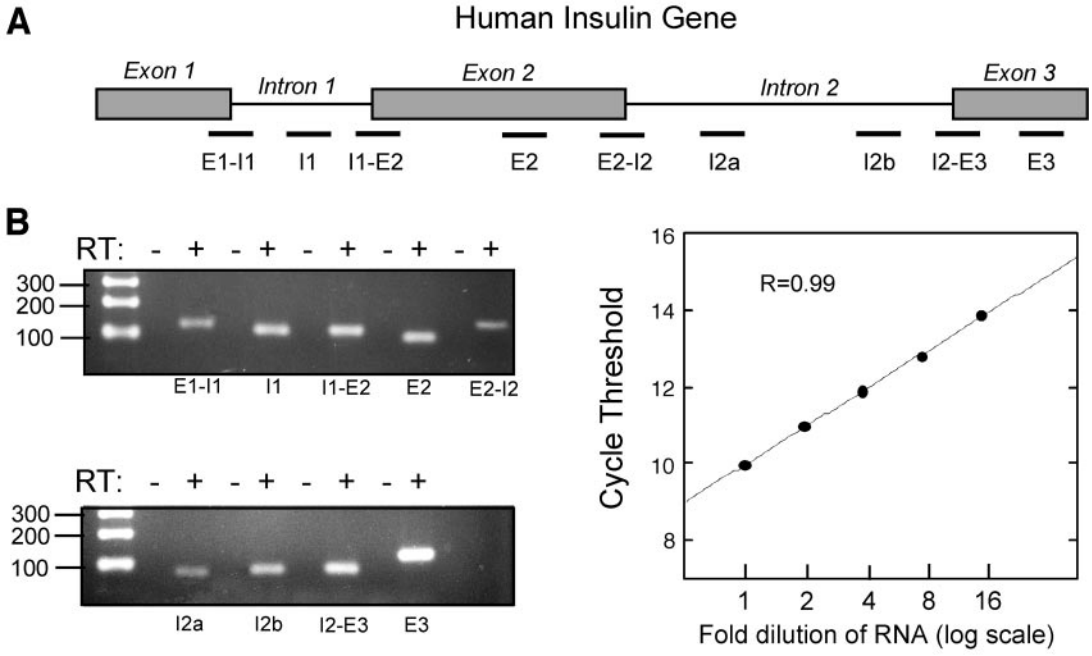


**FIG. 1. Structure of the human insulin gene and validation of primer sets. *A*: Schematic representation of the human insulin gene, indicating positions of each amplicon (solid lines) relative to the introns and exons of the gene. *B*: 2% agarose gel showing homogeneity and expected molecular weight of each amplicon using reverse-transcribed human islet RNA as template. *C*: Real-time PCR threshold cycles demonstrating linearity of amplification for primer set E3 from serial twofold dilutions of human islet reversed-transcribed RNA. R, correlation coefficient for the data modeled as a linear relationship.**

TABLE 2
Real-time RT-PCR threshold cycles ($C_t$) for insulin transcript species in human islets and their estimated relative abundance

| mRNA species | $C_t$ (± SE)* | Approximate relative abundance† |
|---|---|---|
| Human intron 1–exon 1 (I1-E1) | 19.6 ± 1.1 | 0.006 |
| Human intron 1 (I1) | 19.4 ± 1.2 | 0.007 |
| Human intron 1–exon 2 (I1-E2) | 19.7 ± 1.0 | 0.006 |
| Human exon 2 (E2) | 12.3 ± 0.3 | 1 |
| Human exon 2–intron 2 (E2-I2) | 23.4 ± 0.1 | 0.0005 |
| Human intron 2a (I2a) | 22.7 ± 1.7 | 0.0007 |
| Human intron 2b (I2b) | 23.5 ± 0.9 | 0.0004 |
| Human intron 2–exon 3 (I2-E3) | 23.4 ± 0.5 | 0.0005 |
| Human exon 3 (E3) | 12.6 ± 0.3 | 0.80 |

*To correct for variations in input RNA, $C_t$ values were normalized to the actin mRNA $C_t$ in each sample, which was ~15; †relative abundance was calculated using the following formula: $2^{[CT(E2\ mRNA)\ -\ CT(mRNA\ of\ interest)]}$. As noted under RESEARCH DESIGN AND METHODS, RT-PCR primers for insulin mRNA and pre-mRNA were equally efficient.

For glucose induction studies, ~100 islets (human or mouse) per treatment group were cultured in medium containing 2.5 mmol/l glucose for 4 h and then switched to fresh medium containing 2.5, 7, or 25 mmol/l glucose for up to 48 h. For the RNA decay experiments, islets were cultured for 30 min in either 2.5 or 25 mmol/l glucose and subsequently treated with 100 μg/ml of actinomycin D (Sigma). At the indicated times, islets were washed once with PBS and processed for RNA isolation using the RNeasy kit (Qiagen) according to the manufacturer's instructions.

**Glucose-stimulated insulin secretion.** Insulin secretion was measured separately for each human islet preparation as previously described (19). We washed ~50 islets per condition, incubated them in Krebs-Ringer HEPES-buffered solution for 1 h at 37°C, and then placed them in Krebs-Ringer HEPES-buffered solution containing 2.5 or 25 mmol/l glucose for 1 h. Insulin released into the medium was assayed using a two-site immunospecific enzyme-linked immunosorbent assay (Alpco Diagnostics).

**Real-time RT-PCR.** Total RNA (5 μg) from islets was reverse-transcribed at 37 °C for 1 h using 15 μg random hexamers, 0.5 mm dNTPs, 5× first-strand buffer, 0.01 mol/l dithiothreitol, and 200 units of MMLV (Moloney murine leukemia virus) reverse transcriptase (Invitrogen) in a final reaction volume of 20 μl. SYBR Green I–based real-time RT-PCR was performed as described previously (20) using the following thermal cycling protocol for 50 cycles: 95°C for 15 s followed by 62°C for 1 min. All human and mouse RT-PCR primer sets used to amplify the insulin gene in these studies are presented in Table 1. Primer sets for the human and mouse II insulin genes were designed using Vector NTI Suite software (Invitrogen). Forward and reverse primers used to amplify β-actin message in human and mouse islets were 5′-AGGTCATCACT ATTGGCAACGA-3′ and 5′-CACTTCATGATGGAATTGAATGTAGTT-3′.

Amplified product from each PCR was 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 ($C_t$) methodology (21) was used to calculate relative quantities of mRNA products from each sample; all samples were corrected for total input RNA by normalizing $C_t$ values to the $C_t$ value of β-actin message. These corrections were verified by quantitation of total RNA in each sample by fluorescence using RiboGreen dye (Molecular Probes). All primer pairs displayed equal efficiency for amplification of target DNA, which was determined by testing each pair on genomic DNA from human (HEK293) or mouse (NIH3T3) cell lines.

**Quantitative chromatin immunoprecipitation assay.** A total of ~300 islets were fixed in 1% formaldehyde for 15 min, sonicated to shear DNA fragments in the range 800–2,000 bp, and subjected to chromatin immunoprecipitation as detailed previously (21). Chromatin immunoprecipitation assays were performed on at least three independent islet isolations. For each isolation, samples were quantitated in triplicate by SYBR Green I–based real-time PCR using forward and reverse primer sequences for either the mouse or the human insulin genes. Forward and reverse primers used to amplify the proximal mouse gene were 5′-TCAGCCAAAGATGAAGAAGGTCT C-3′ and 5′-TCCAAACACTTGCCTGGTGC-3′, respectively, and those for the human gene were 5′-CCAGCCGCAGCCTTTGTGAA-3′ (forward) and 5′-AG CAATGGGCGGTTGGCTCA-3′ (reverse). Rabbit polyclonal antibodies to acetylated H3 and acetylated H4 were from Upstate Biotechnology, and monoclonal antibody against the COOH-terminal heptapeptide repeat domain of RNA polymerase II was purchased from Covance.

## RESULTS

**Quantitation of human insulin mRNA and pre-mRNAs.** To measure human insulin mRNA and pre-mRNA species, we designed multiple PCR primer sets that amplified segments of exons, introns, or intron-exon boundaries of the human insulin gene coding region, as shown in Table 1 and Fig. 1*A*. Primer sets that amplify segments of exons only (sets E2 and E3) (Fig. 1*A*) measure both spliced and unspliced mRNA (i.e., total mRNA). Those that amplify segments of introns only or intron-exon boundaries measure unspliced mRNA species (i.e., pre-mRNAs). Figure 1*B* shows that each primer set amplifies a homogenous product of expected molecular weight when reversed-transcribed human islet RNA is used as a template. These primers were then used for SYBR Green I–based real-time PCR. Figure 1*C* shows that primer set E3 amplified product in a quantitative manner over the narrow range of template concentrations observed in our studies, thereby demonstrating the linearity of our real-time PCR assay. In the absence of RT, $C_t$ values for real-time PCR were ~15 cycles higher than in the presence of RT, indicating that genomic DNA contamination was only ~0.003% ($2^{-15} \times 100$). Data for all of the other primer sets used in this study (including those amplifying β-actin message) were similar (data not shown).

Table 2 shows the relative abundance of each mRNA and pre-mRNA species in cultured human islets maintained in 2.5 mmol/l glucose. The table displays the fraction of each amplified fragment relative to total mRNA (i.e., relative to the quantity of the fragment amplified by primer set E2). Notably, pre-mRNAs containing intron 1 (amplified by primer sets I1-E1, I1, and I1-E2) are ~150-fold less abundant than total RNA, whereas intron 2–containing pre-mRNAs (amplified by primer sets E2-I2, I2a,

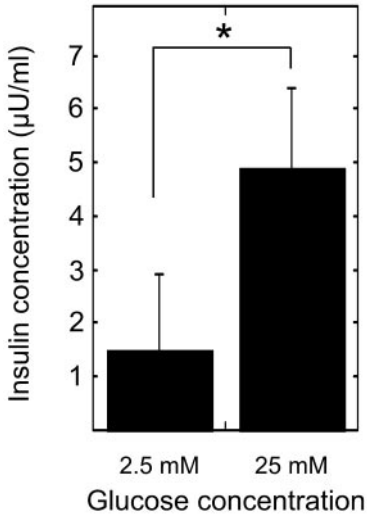


**FIG. 2. Glucose-stimulated insulin secretion in human islets. Human islets were incubated in either 2.5 or 25 mmol/l glucose for 30 min, after which insulin concentration was measured in the medium using enzyme-linked immunosorbent assay. Data represent the average for 14 different human islet preparations. *Statistically significant ($P < 0.05$).**

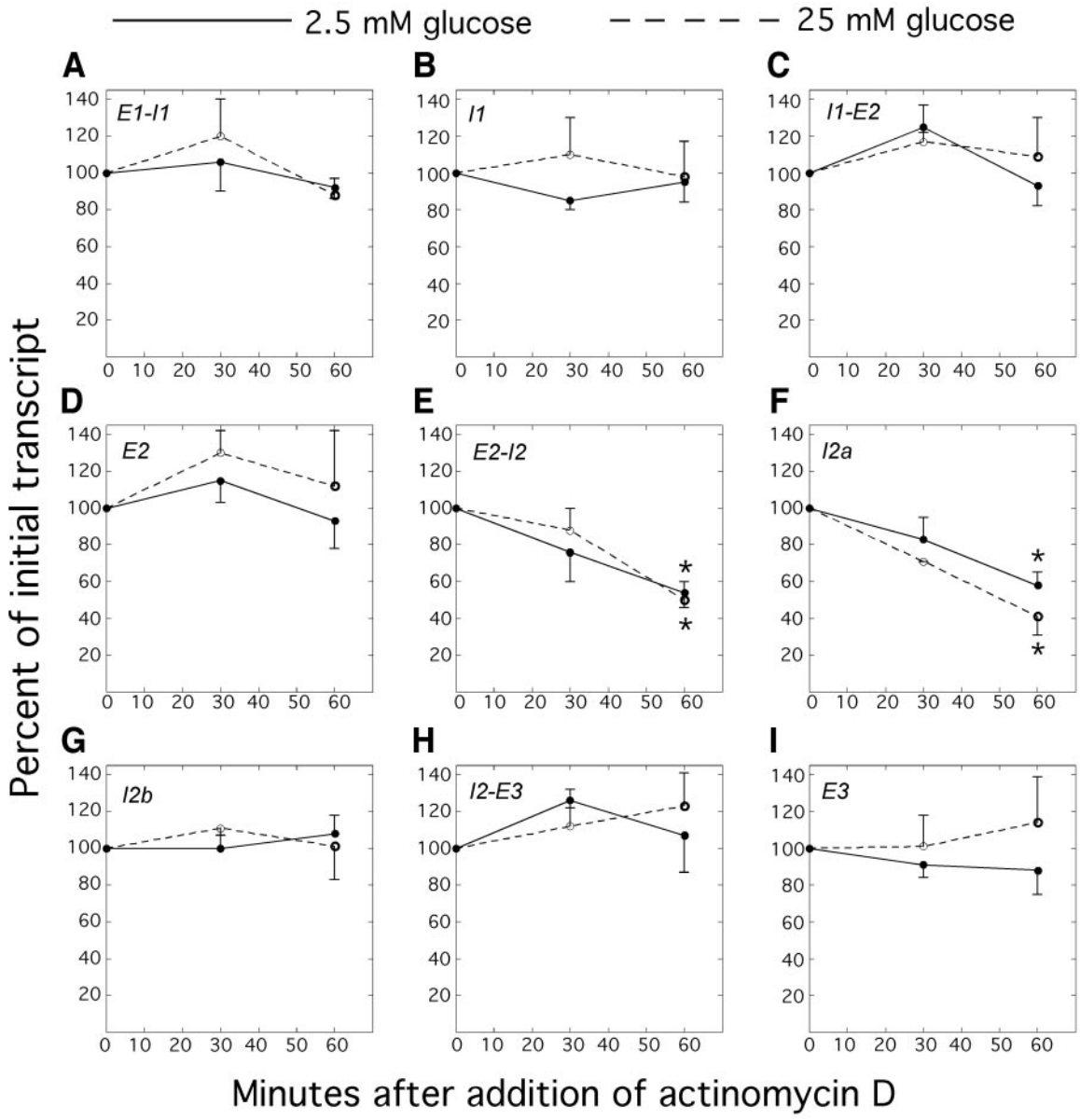


**FIG. 3. Human insulin transcript decay rates. Human islets exposed to either 2.5 or 25 mmol/l glucose were incubated with 100 μg/ml actinomycin D for 0, 30, and 60 min. Reverse-transcribed RNA was subjected to real-time PCR quantitation using each of the nine primer sets shown in Fig. 1*A*: E1-I1 (*A*), I1 (*B*), I1-E2 (*C*), E2 (*D*), E2-I2 (*E*), I2a (*F*), I2b (*G*), I2-E3 (*H*), and E3 (*I*). *Values of transcript at 60 min significantly different ($P < 0.05$) from baseline ($t = 0$) transcript levels.**

I2b, and I2-E3) are 1,500- to 2,500-fold less abundant. The differences in relative abundance reported here are not secondary to differences in amplification efficiencies of the primer sets because all primer sets amplified target sequences from human genomic DNA (HEK293 cells) with comparable threshold values (data not shown). Taken together, the data in Fig. 1 and Table 2 indicate that our assay quantitatively detects unprocessed insulin mRNA species (pre-mRNAs) and that these represent a small but measurable fraction of total insulin message in human islets. Because unprocessed pre-mRNAs represent a very small fraction of total insulin mRNA at steady state, for the discussion that follows, mature (i.e., fully processed) mRNA can be considered as effectively equivalent to total mRNA measured by primer sets E2 or E3.

**Biologic decay of human insulin transcripts.** To first verify that the human islets we obtained from each preparation were functional, we assessed glucose-stimulated insulin secretion by static incubation assays. Raising glucose in the medium from 2.5 to 25 mmol/l led to an approximately threefold increase in insulin concentration in the medium after 30 min (Fig. 2); these data are similar to those reported in the literature for adequate human islet preparations (22). We next sought to estimate the biologic half-lives of mature insulin mRNA and pre-mRNAs. To do this, we exposed human islets to 100 μg/ml actinomycin D to inhibit transcriptional elongation and then quantitated transcript levels at 30 and 60 min after exposure. Figure 3 shows that at low (2.5 mmol/l) glucose concentrations, pre-mRNA containing intron 1 (Fig. 3*A–C*) as well as mature mRNA (Fig. 3*D* and *I*) did not show significant changes in abundance after 60 min of exposure to actinomycin D. These data suggest that mature and intron 1–containing mRNAs are relatively long-lived species, with half-lives well in excess of 60 min, and are consistent with observations made in mouse islets (20,23). By con-

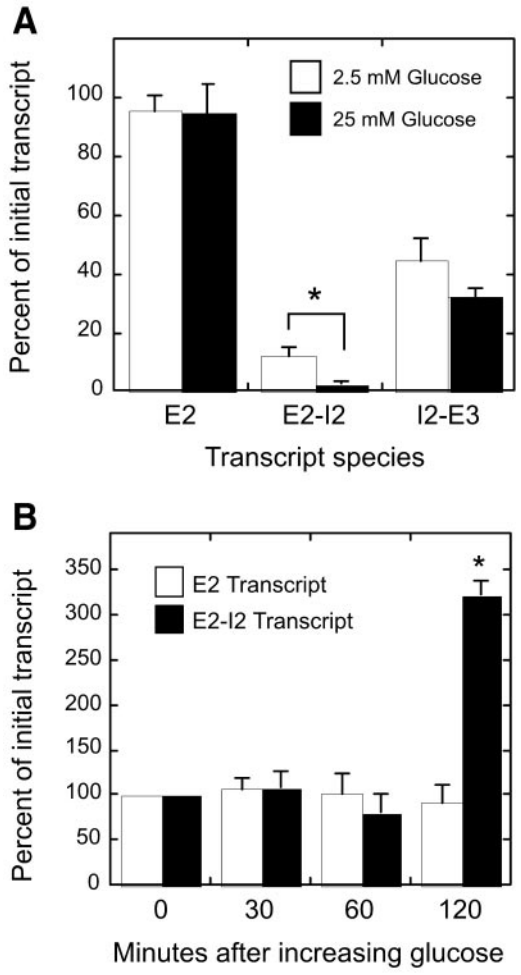


**FIG. 4. Insulin transcript decay rates and glucose-stimulated insulin transcription in mouse islets. *A*: Mouse islets exposed to either 2.5 or 25 mmol/l glucose were incubated with 100 μg/ml actinomycin D for 60 min and harvested for measurement of the transcript species indicated by real-time PCR (and compared with levels at $t = 0$ min). *Statistically different ($P < 0.05$). *B*: Glucose concentration in the medium of cultured mouse islets was increased from 2.5 to 25 mmol/l glucose for the times indicated and harvested for measurement of the transcript species indicated by real-time PCR. *Statistically different ($P < 0.05$) than the value at $t = 0$ min.**

trast, concentrations of pre-mRNA containing the 5′ portion of intron 2 (Fig. 3*E* and *F*) decreased to 40–50% of initial levels after 60 min, suggesting that this region of the intron exhibits a relatively short half-life (~60 min) owing to rapid exon 2–intron 2 boundary splicing. Pre-mRNA species harboring the middle and 3′ portions of intron 2, however, did not diminish during the 60-min incubation with actinomycin D (Fig. 3*G* and *H*). The data in Fig. 3 were unchanged when actinomycin D concentrations were increased to 300 μg/ml (data not shown), implying that maximal inhibition of transcription was achieved in these studies.

**Effect of glucose on insulin gene transcript decay.** To determine the effect of glucose on insulin transcript decay, we repeated the actinomycin D studies at 25 mmol/l glucose. Within the 60-min time frame of these experiments, the higher glucose concentration had no detectable effect on the decay of any human insulin mRNA or pre-mRNAs measured (Fig. 3). These results stand in striking contrast to the mouse insulin II gene (the gene that is homologous to the human insulin gene). As shown in Fig. 4*A*, whereas levels of mature mouse insulin mRNA (E2) did not change during a 60-min incubation with actinomycin D, mouse intron 2–containing pre-mRNAs (E1-I2 and I2-E2) decreased substantially under low glucose conditions (to 12 and 44%, respectively, of initial levels). In contrast to human insulin, the disappearance of both of these pre-mRNA species was accelerated at high (25 mmol/l) glucose during the same 60-min incubation (Fig. 4*A*). Notably, like the human insulin gene, the 3′ end of the mouse intron 2 (I2-E3) appears to exhibit slower decay kinetics than the 5′ end (E2-I2).

**Acute effect of glucose on insulin gene transcription.** The low abundance and relatively short half-life of the 5′ intron 2–containing pre-mRNA (E2-I2) in human islets suggested to us that measurement of this transcript would reflect more accurately any acute changes to insulin transcription that occurs in response to various transcriptional modulators. To test this possibility, we subjected human islets to an acute increase in glucose (from 2.5 to 25 mmol/l) and measured mature mRNA (E2) and several pre-mRNAs (I1-E2, E2-I2, and I2-E3). As shown in Fig. 5*A*, the increase in glucose to 25 mmol/l caused no measurable change in mature mRNA levels, even after 120 min. By contrast, pre-mRNA species showed significant increases (~1.5-fold) within 60–120 min in human islets, with the E2-I2 transcript declining to baseline levels by 120 min (Fig. 5*B–D*). We observed a similar rapid increase in the mouse E2-I2 transcript (within 120 min) in response to glucose (Fig. 4*B*). As with human islets, the mouse mature mRNA transcript (E2) did not show any changes during the 120-min time course (Fig. 4*B*).

Because 60 min was required to observe an increase in the E2-I2 transcript in human islets in response to glucose, it is possible that this "delay" either *1*) reflects the actual time required for transcript initiation in response to glucose or *2*) simply reflects the time required for transcript to accumulate to measurable quantities above background levels. To distinguish these possibilities, we subjected human islets to 25 mmol/l glucose for 30 min and subsequently performed chromatin immunoprecipitation assays to assess histone H3 and H4 acetylation (both markers of acute gene activation) (24) and RNA polymerase II recruitment at the proximal insulin promoter region. As shown in Fig. 6, we observed a sixfold increase in H4 acetylation (but no increase in H3 acetylation) at the insulin promoter within 30 min of exposure to elevated glucose. Concurrent with this increase in H4 acetylation, we observed a twofold increase in the occupancy of RNA polymerase II at the insulin promoter. These findings strongly suggest that glucose appears to exert a rapid (≤30 min) increase in insulin transcription, but that measurable increases in pre-mRNA are delayed by at least 60 min.

**Chronic effect of glucose on insulin gene transcription.** To measure the chronic effects of glucose on insulin transcription in human islets, we incubated human islets in either 7 or 25 mmol/l glucose for up to 48 h. As shown in Fig. 7*A*, an increase in mature mRNA (E2 transcript) was not observed until 48 h, consistent with prior studies by Muller et al. (17). However, all other pre-mRNA species showed gradual increases throughout the 48-h incubation (Fig. 7*B–D*), suggesting that the more chronic incubation in elevated glucose caused a sustained increase in insulin transcription that is only evident when measuring species that have lower abundance.

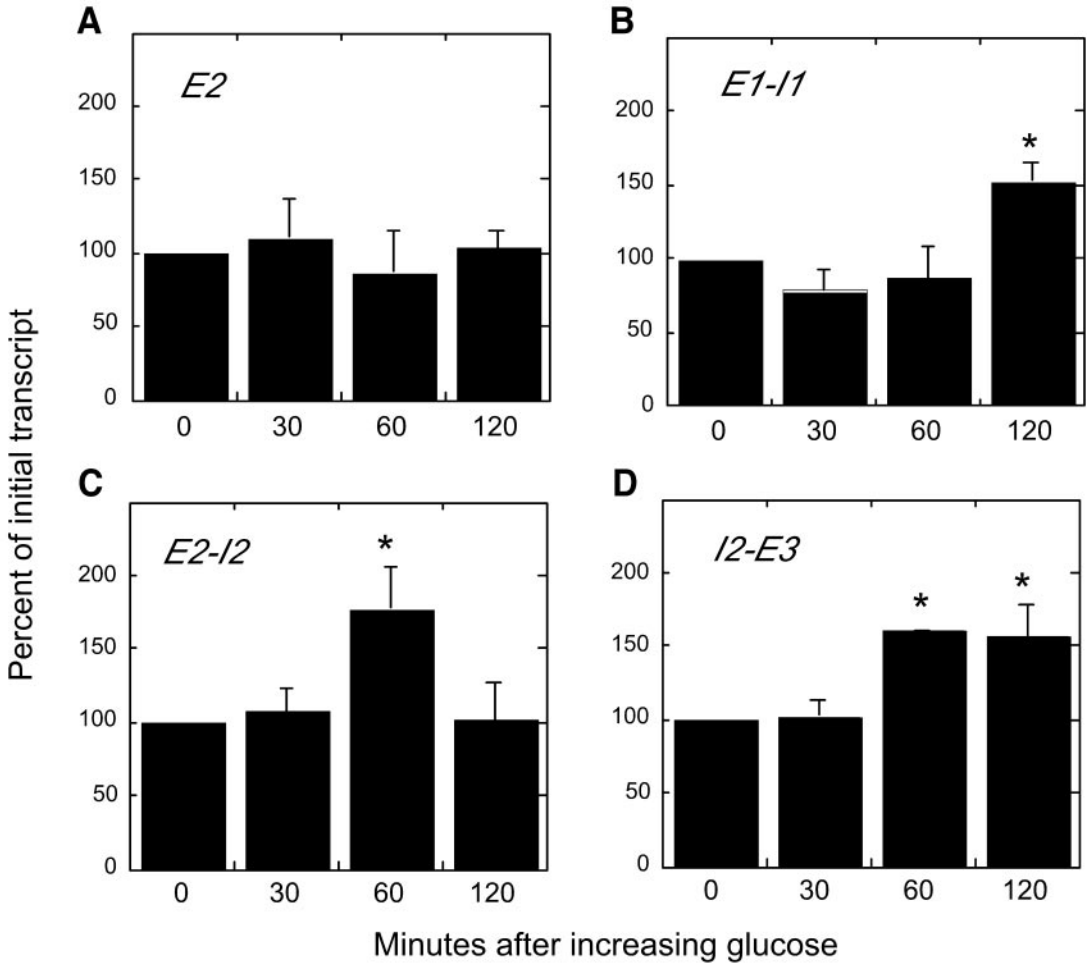


**FIG. 5. Acute glucose-stimulated insulin transcription in human islets. Glucose concentration in the medium of cultured human islets was increased from 2.5 to 25 mmol/l glucose for the times indicated. Reverse-transcribed total RNA from the human islets was then subjected to real-time PCR using primer set E2 (*A*), E1-I1 (*B*), E2-I2 (*C*), and I2-E3 (*D*) to measure mature mRNA and pre-mRNA. *Statistically different ($P < 0.05$) than the value at $t = 0$ min.**

## DISCUSSION

Recent interest in the therapeutic use of stem cells and other transdifferentiated cell types for the treatment of diabetes has necessitated the need to better define the biology of gene transcription in human islets (12). To date, however, the vast majority of these studies have focused on rodent cell types using transfected promoter reporters. Here, we present studies on the glucose regulation of insulin gene transcription and transcript processing in human pancreatic islets. Our data revealed that both total (or mature) mRNA and intron 1–containing pre-mRNA appear to have remarkably long rates of decay (>60 min) in the cell. These findings are similar to those for the mouse insulin genes, wherein half-lives of these species in tumorigenic cell lines are reported to be well in excess of 24 h (10,25–27). By contrast, intron 2–containing species exhibit a strikingly lower abundance (~10- to 20-fold lower than intron 1–containing species and 1,500- to 2,500-fold lower than mature mRNA) and a more variable half-life.

Our observation that the 5′ portion of intron 2 has a shorter half-life than the 3′ portion is consistent with the accepted model of intron splicing, where splicing proceeds in a 5′ to 3′ direction (28). Splicing of pre-mRNA introns is performed by the spliceosome, which removes introns through a series of ordered RNA-RNA, RNA-protein, and protein-protein interactions (28,29). Our ability to measure distinct species of intron 2–containing pre-mRNAs provides insight into the kinetics of splicing of intron 2. Assuming that rates of decay as determined here reflect processing rates for pre-mRNAs, then our data suggest two possibilities. The first is that splicing of intron 2 is a continuous process during the course of transcription, but the processing of the 3′ end is sufficiently delayed such that detection of the decay of this species was outside of the time frame of our actinomycin experiments. A second possibility is that intron 2 is spliced in a multistep process, with the 5′ region being spliced first, and that the 3′ intron 2 species described here represents a cytoplasmic mRNA species that remains in its immature form. This second possibility would require the presence of an intermediate splicing recognition site within intron 2. In this regard, Shalev et al. (30) identified a cryptic splice site within intron 1 of the human insulin gene that leads to the generation of an alternatively spliced form of insulin message with the retention of 26 bp of intron 1. Although this region is not within the coding region, studies in vitro suggested increased translational efficiency of this alternative species (30). Such alternative and/or intermediate splice sites have not been previously identified within intron 2.

The importance of glucose in the β-cell appears to

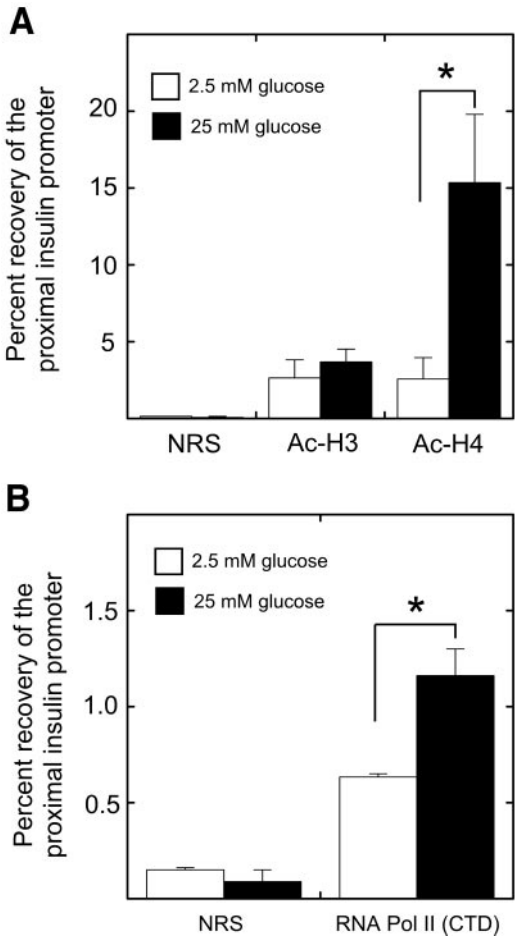


**FIG. 6. Histone acetylation and RNA polymerase II occupancy at the human insulin gene. Human islets were incubated in either 2.5 or 25 mmol/l glucose for 30 min and subjected to the chromatin immunoprecipitation assay as detailed in RESEARCH DESIGN AND METHODS. Real-time RT-PCR was used to quantitate recovery of the proximal insulin promoter, and results are expressed as the percent recovery of the gene fragment relative to input. *A*: Chromatin immunoprecipitation using normal rabbit serum (NRS) or antibodies to acetylated H3 (Ac-H3) and acetylated H4 (Ac-H4). *B*: Chromatin immunoprecipitation using normal rabbit serum or antibodies to RNA polymerase (Pol) II. *Statistically different ($P < 0.05$). CTD, COOH-terminal heptapeptide repeat domain.**

extend beyond its well-known role as a stimulator of insulin granule release. Glucose is believed to enhance insulin gene transcription, stabilize mature insulin mRNA, promote its translation to new protein, and enhance posttranslational proinsulin processing (2,10,25,31). These effects of glucose ultimately serve to maintain appropriate stores of insulin after repeated glucose challenges. In mouse islets, whereas glucose extends the half-life of mature insulin message, it appears to decrease the half-life of insulin pre-mRNAs (by up to twofold) through mechanisms as yet undefined (this report and 20,23). Presumably, this effect of glucose ensures more rapid processing of pre-mRNAs to the mature product under conditions of greater insulin demand. Our data, however, reveal no significant effect of glucose on human insulin pre-mRNA stability (at least those species containing intron 2).

Our findings of the low abundance of intron 2–containing pre-mRNAs and their more rapid turnover rate relative to mature mRNAs (particularly of the 5′ end) suggest that this species may reflect most accurately the short-term changes in insulin transcription that occur in response to various stimuli or inhibitors. Our data strongly support this notion because acute elevations in glucose resulted in a nearly twofold rise in intron 2–containing pre-mRNA levels within 60 min and a return to baseline levels at 120 min, but no change in mature mRNA levels. Other pre-mRNA species showed a similar increase at 60 min, but no decrease at 120 min (presumably because these species exhibit half-lives >60 min). The lack of an observable effect on mature mRNA levels is likely a consequence of the enormous abundance of this species in islets (Table 2).

Notwithstanding the delays observed in the accumulation of pre-mRNA in response to glucose in both species, we believe that the effect of glucose on insulin gene transcription occurs very quickly because we observed an increase in RNA polymerase II recruitment at the human insulin gene within 30 min of exposure to elevated glucose. Preceding or coinciding with this increase in RNA polymerase II recruitment, we observed an acute change in the structure of chromatin (as assessed by an increase in H4 acetylation) at the insulin gene. Histone acetylation is known to increase nucleosome mobility and thereby enhance chromatin accessibility to transcriptional regulators (32,33). Precisely how this increase in acetylation occurs is subject to some speculation, although studies in rodent insulinoma cells suggest that recruitment of histone acetyltransferases such as p300 or P/CAF (p300/cAMP response element-binding protein–associating factor) by Pdx-1, NeuroD1, and/or E47 may underlie this phenomenon (8,34,35).

Given the low abundance of pre-mRNAs, it is unlikely that the early increase in transcription would translate to measurable increases in insulin protein. Instead, the increases seen in the long term are likely more relevant in this regard. More chronic exposure of human islets to glucose eventually revealed an increase in mature mRNA, but this increase required almost 48 h. These findings are consistent with a very recent report by Muller et al. (17). Although this increase in mRNA levels at 48 h could be secondary to enhanced stability, we propose that a component of this increase is likely a result of a sustained increase in transcriptional rate because pre-mRNA levels were also increased throughout the 48-h incubation. Thus, measurement of pre-mRNAs allowed us to detect early changes to gene transcription that would have otherwise been missed. It is noteworthy that the 5′ intron 2–containing transcript decreased after 2-h incubation and then increased again at 8 h and remained sustained thereafter. This finding is reminiscent of the biphasic insulin secretory response of the β-cell to constant glucose infusions, and it supports the proposal by Muller et al. (17) that insulin signaling in human islets may impact insulin gene transcription.

Taken together, our data identify the abundance and relative lifetimes of insulin transcripts (mRNAs and pre-mRNAs) in human islets, suggest a preference for the order of intron splicing in human and mouse islets, and underscore the utility and necessity of measuring pre-mRNA species to detect acute changes in transcriptional rates of the insulin gene in response to glucose. Importantly, we observed significant differences in the processing rates of the insulin gene pre-mRNA in human islets compared with mouse islets, thereby underscoring the necessity for continued efforts to define better the biology of human islets in the normal and diseased state.

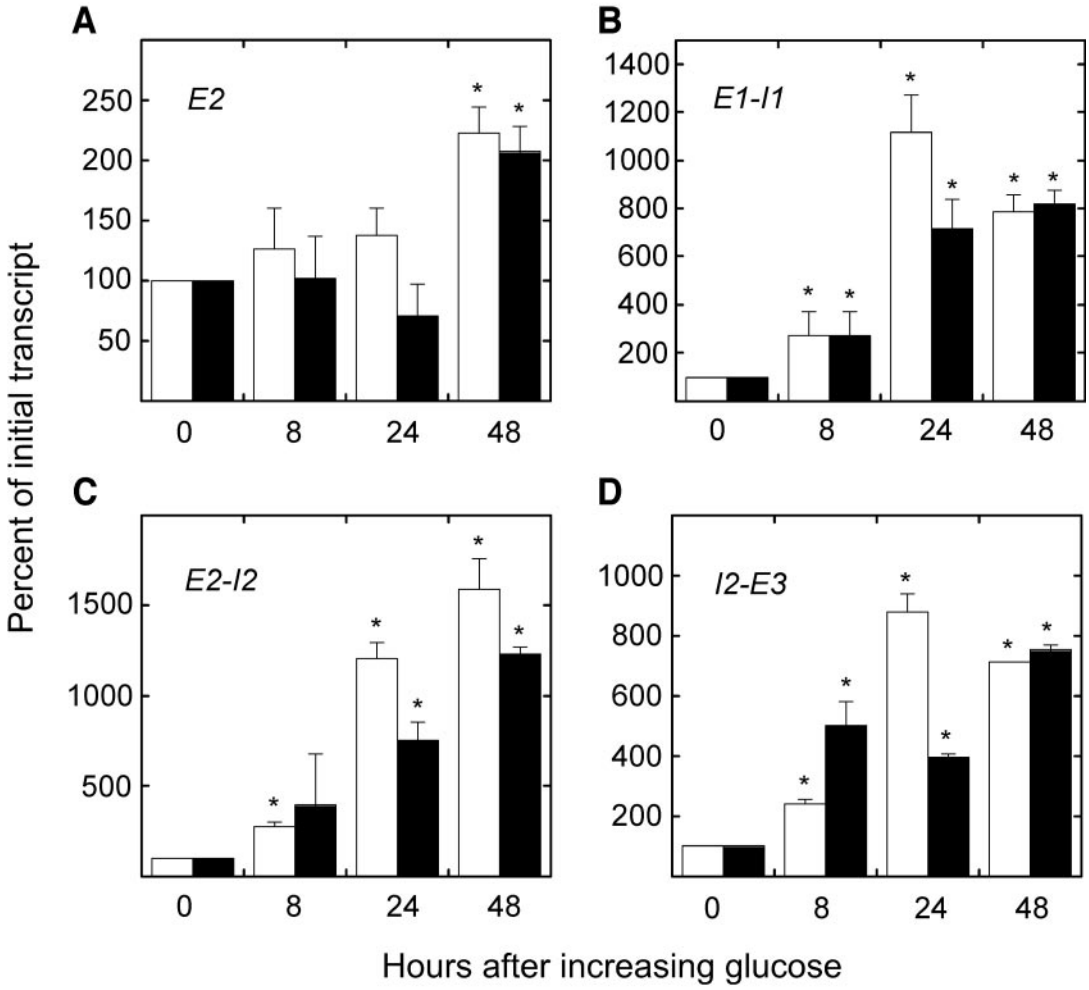


**FIG. 7. Chronic glucose-stimulated insulin transcription in human islets. Glucose concentration in the medium of cultured human islets was increased from 2.5 to either 7 mmol/l glucose □ or 25 mmol/l ■ glucose for the times indicated. Reverse-transcribed total RNA from the human islets was then subjected to real-time PCR using primer set E2 (*A*), E1-I1 (*B*), E2-I2 (*C*), and I2-E3 (*D*) to measure mature mRNA and pre-mRNA. *Statistically different ($P < 0.05$) than the value at $t = 0$ min.**


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## REFERENCES


1. German MS, Wang J: The insulin gene contains multiple transcriptional elements that respond to glucose. *Mol Cell Biol* 14:4067–4075, 1994
2. Wicksteed B, Alarcon C, Briaud I, Lingohr MK, Rhodes CJ: Glucose-induced translational control of proinsulin biosynthesis is proportional to preproinsulin mRNA levels in islet beta-cells but not regulated via a positive feedback of secreted insulin. *J Biol Chem* 278:42080–42090, 2003
3. Docherty HM, Hay CW, Ferguson LA, Barrow J, Durward E, Docherty K: Relative contribution of PDX-1, MafA and E47/beta2 to the regulation of the human insulin promoter. *Biochem J* 389:813–820, 2005
4. Khoo S, Griffen SC, Xia Y, Baer RJ, German MS, Cobb MH: Regulation of insulin gene transcription by ERK1 and ERK2 in pancreatic beta cells. *J Biol Chem* 278:32969–32977, 2003
5. Lawrence MC, McGlynn K, Park BH, Cobb MH: ERK1/2-dependent activation of transcription factors required for acute and chronic effects of glucose on the insulin gene promoter. *J Biol Chem* 280:26751–26759, 2005
6. Chakrabarti SK, Francis J, Ziesmann SM, Garmey JC, Mirmira RG: Covalent histone modifications underlie the developmental regulation of insulin gene transcription in pancreatic beta cells. *J Biol Chem* 278:23617–23623, 2003
7. Francis J, Chakrabarti SK, Garmey JC, Mirmira RG: Pdx-1 links histone H3-Lys-4 methylation to RNA polymerase II elongation during activation of insulin transcription. *J Biol Chem* 280:36244–36253, 2005
8. Mosley AL, Ozcan S: Glucose regulates insulin gene transcription by hyperacetylation of histone H4. *J Biol Chem* 278:19660–19666, 2003
9. Mosley AL, Corbett JA, Ozcan S: Glucose regulation of insulin gene expression requires the recruitment of p300 by the beta-cell-specific transcription factor Pdx-1. *Mol Endocrinol* 18:2279–2290, 2004
10. Tillmar L, Carlsson C, Welsh N: Control of insulin mRNA stability in rat pancreatic islets: regulatory role of a 3′-untranslated region pyrimidine-rich sequence. *J Biol Chem* 277:1099–1106, 2002
11. Cabrera O, Berman DM, Kenyon NS, Ricordi C, Berggren PO, Caicedo A: The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. *Proc Natl Acad Sci U S A* 103:2334–2339, 2006
12. Hay CW, Docherty K: Comparative analysis of insulin gene promoters: implications for diabetes research. *Diabetes* 55:3201–3213, 2006
13. Melloul D, Marshak S, Cerasi E: Regulation of insulin gene transcription. *Diabetologia* 45:309–326, 2002
14. Le Lay J, Stein R: Involvement of PDX-1 in activation of human insulin gene transcription. *J Endocrinol* 188:287–294, 2006

15. Le Lay J, Matsuoka TA, Henderson E, Stein R: Identification of a novel PDX-1 binding site in the human insulin gene enhancer. *J Biol Chem* 279:22228–22235, 2004
16. Sander M, Griffen SC, Huang J, German MS: A novel glucose-responsive element in the human insulin gene functions uniquely in primary cultured islets. *Proc Natl Acad Sci U S A* 95:11572–11577, 1998
17. Muller D, Huang GC, Amiel S, Jones PM, Persaud SJ: Identification of insulin signaling elements in human beta-cells: autocrine regulation of insulin gene expression. *Diabetes* 55:2835–2842, 2006
18. Yang Z, Chen M, Ellett JD, Fialkow LB, Carter JD, Nadler JL: The novel anti-inflammatory agent lisofylline prevents autoimmune diabetic recurrence after islet transplantation. *Transplantation* 77:55–60, 2004
19. Yang Z, Chen M, Ellett JD, Carter JD, Brayman KL, Nadler JL: Inflammatory blockade improves human pancreatic islet function and viability. *Am J Transplant* 5:475–483, 2005
20. Iype T, Francis J, Garmey JC, Schisler JC, Nesher R, Weir GC, Becker TC, Newgard CB, Griffen SC, Mirmira RG: Mechanism of insulin gene regulation by the pancreatic transcription factor Pdx-1: application of pre-mRNA analysis and chromatin immunoprecipitation to assess formation of functional transcriptional complexes. *J Biol Chem* 280:16798–16807, 2005
21. Chakrabarti SK, James JC, Mirmira RG: Quantitative assessment of gene targeting in vitro and in vivo by the pancreatic transcription factor, Pdx1: importance of chromatin structure in directing promoter binding. *J Biol Chem* 277:13286–13293, 2002
22. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV: Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 343:230–238, 2000
23. Wang J, Shen L, Najafi H, Kolberg J, Matschinsky FM, Urdea M, German M: Regulation of insulin preRNA splicing by glucose. *Proc Natl Acad Sci U S A* 94:4360–4365, 1997
24. Turner BM: Histone acetylation and an epigenetic code. *Bioessays* 22:836–845, 2000
25. Welsh M, Nielsen DA, MacKrell AJ, Steiner DF: Control of insulin gene expression in pancreatic beta-cells and in an insulin-producing cell line, RIN-5F cells. II. Regulation of insulin mRNA stability. *J Biol Chem* 260:13590–13594, 1985
26. Ritz-Laser B, Meda P, Constant I, Klages N, Charollais A, Morales A, Magnan C, Ktorza A, Philippe J: Glucose-induced preproinsulin gene expression is inhibited by the free fatty acid palmitate. *Endocrinology* 140:4005–4014, 1999
27. Kelpe CL, Moore PC, Parazzoli SD, Wicksteed B, Rhodes CJ, Poitout V: Palmitate inhibition of insulin gene expression is mediated at the transcriptional level via ceramide synthesis. *J Biol Chem* 278:30015–30021, 2003
28. Sharp PA: Split genes and RNA splicing. *Cell* 77:805–815, 1994
29. Baralle D, Baralle M: Splicing in action: assessing disease causing sequence changes. *J Med Genet* 42:737–748, 2005
30. Shalev A, Blair PJ, Hoffmann SC, Hirshberg B, Peculis BA, Harlan DM: A proinsulin gene splice variant with increased translation efficiency is expressed in human pancreatic islets. *Endocrinology* 143:2541–2547, 2002
31. Nielsen DA, Welsh M, Casadaban MJ, Steiner DF: Control of insulin gene expression in pancreatic beta-cells and in an insulin-producing cell line, RIN-5F cells. I. Effects of glucose and cyclic AMP on the transcription of insulin mRNA. *J Biol Chem* 260:13585–13589, 1985
32. Anderson JD, Lowary PT, Widom J: Effects of histone acetylation on the equilibrium accessibility of nucleosomal DNA target sites. *J Mol Biol* 307:977–985, 2001
33. Francis J, Babu DA, Deering TG, Chakrabarti SK, Garmey JC, Evans-Molina C, Taylor DG, Mirmira RG: Role of chromatin accessibility in the occupancy and transcription of the insulin gene by the pancreatic and duodenal homeobox factor 1. *Mol Endocrinol* 20:3133–3145, 2006
34. Qiu Y, Guo M, Huang S, Stein R: Insulin gene transcription is mediated by interactions between the p300 coactivator and PDX-1, BETA2, and E47. *Mol Cell Biol* 22:412–420, 2002
35. Qiu Y, Guo M, Huang S, Stein R: Acetylation of the BETA2 transcription factor by p300-associated factor is important in insulin gene expression. *J Biol Chem* 279:9796–9802, 2004