Control of Insulin mRNA Stability in Rat Pancreatic Islets

REGULATORY ROLE OF A 3'-UNTRANSLATED REGION PYRIMIDINE-RICH SEQUENCE

Received for publication, August 29, 2001, and in revised form, November 1, 2001
Published, JBC Papers in Press, November 5, 2001, DOI 10.1074/jbc.M108340200

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Stabilization of insulin mRNA in response to glucose is a significant component of insulin production, but the mechanisms governing this process are unknown. We presently observe that insulin mRNA is a highly abundant messenger and that the content of this mRNA is mainly controlled by changes in messenger stability. We also demonstrate specific binding of the polypyrimidine tract-binding protein to a pyrimidine-rich sequence located in the 3'-untranslated region (3'-UTR) of insulin mRNA. This binding was increased in vitro by dithiothreitol and in vivo by glucose. Inhibition of polypyrimidine tract-binding protein binding to the pyrimidine-rich sequence by mutation of the core binding site resulted in a destabilization of a reporter gene mRNA. Thus, glucose-induced binding of polypyrimidine tract-binding protein to the 3'-UTR of insulin mRNA could be a necessary event in the control of insulin mRNA levels.

Glucose is the main regulator of insulin biosynthesis (1). Besides controlling insulin biosynthesis by modulating protein synthesis initiation and elongation rates (2), glucose also stimulates the production of insulin by increasing insulin mRNA levels (3). This effect is achieved by a selective stimulation of transcriptional and translational mechanisms to the control of insulin mRNA levels is under debate. According to the original view, insulin mRNA is a highly abundant messenger (6), which is not affected by short term glucose challenges (7), and therefore controlled to a large extent by changes in messenger stability (5). The half-life of insulin mRNA was assessed to be 29 h at a low glucose concentration and 77 h at a high glucose concentration (5). Recent observations, however, challenge this view by suggesting that insulin mRNA contents of insulin-producing cells are rapidly and dramatically increased by glucose and that this effect is mediated by stimulation of insulin gene transcription (8–10). In addition, it has also been reported that glucose destabilizes, rather than stabilizes, recently formed insulin mRNA (9).

Very little is known on the mechanisms by which glucose, according to the original view, promotes an increased stability of insulin mRNA. A recent study has identified the 3'-untranslated (3'-UTR) region as a critical region for glucose-mediated control of rat insulin II mRNA stability (11). We have observed that the 3'-UTR of rat insulin mRNA contains a pyrimidine-rich segment directly downstream of the coding sequence (Fig. 1A). Interestingly, similar pyrimidine-rich segments are located in the insulin mRNA 3'-UTR of several mammalian and non-mammalian species including humans (Fig. 1B). Pyrimidine-rich segments are also present in 3'-UTRs of other long-lived messengers, such as α-globin, (I)-collagen, 15-lipoxygenase, and tyrosine hydroxylase (TH) (Ref. 12; Fig. 1A). It appears that the 37–39-kDa polyc(C)-binding protein (PCBP), also known as αCP or hnRNPe, binds to the pyrimidine-rich motifs of these mRNA, thereby assembling a ribonucleoprotein complex, the α-complex, which results in stabilization of the mRNA (13). Another interesting pyrimidine-rich sequence-binding protein is the 56-kDa polypyrimidine tract-binding protein (PTB) (14). As other hnRNP proteins, PTB assists in processing, transport, and translation of mRNAs. However, in insulin-producing MIN6 cells PTB mRNA contents are increased 5-fold by glucose (15). This may indicate that the expression of PTB is under cell-specific control and that PTB contributes to the β-cell phenotype as a glucose-responsive secretory of insulin.

The aim of the present study was first to quantify insulin mRNA in rat pancreatic islets. A high insulin mRNA content would strongly support the original view that insulin mRNA contents are mainly controlled by post-transcriptional mechanisms. Second, we aimed at characterizing the molecular event by which glucose increases insulin RNA stability. We have therefore studied the putative role of the pyrimidine-rich insulin mRNA 3'-UTR in the regulation of insulin mRNA stability.

EXPERIMENTAL PROCEDURES

Materials—The RNA oligonucleotides were from Scandinavian Gene Synthesis AB (Köping, Sweden). The rabbit anti-PCBP serum was a generous gift from the laboratory of Ellie Ehrenfeld. David Helfman’s and Carol Bromstyk’s groups kindly supplied the mouse PTB and rabbit anti-hnRNP K antibodies.

Isolation and Culture of Pancreatic Islets—Adult Sprague-Dawley rats, from a local colony, were used. Islets were isolated by collagenase digestion (17). The islets were cultured in RPMI 1640 supplemented with 10%, Fetal Clone II serum (Hyclone Europe Ltd., Cramlington, UK). The islets were cultured free-floating 5–10 days with the medium changed every second day (16).

Human islets were obtained from the Central Unit of the β-Cell Transplant, Brussels, where the islets were isolated and maintained in culture as described previously (17).

Quantitative Real-time PCR—Total RNA from 20–30 islets was isolated using the Ultraspec® Total RNA Isolation System (Biotech Laboratories, Houston, TX). First strand cDNA was synthesized using random nonamers and avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI). PCR reactions were carried out with the

* This work was supported by grants from the Swedish Medical Research Council (12X-109, 12X-11564, 72P-12995), the Swedish Diabetes Association, the Nordic Insulin Fund, the Juvenile Diabetes Foundation International, and the Family Ernfors Fund. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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Laboratories Inc., Palo Alto, CA).

beled probes were purified on Chroma spin-10 columns (CLONTECH
bacteriophage T4 polynucleotide kinase (Sigma-Aldrich). The radiola-
(5000 Ci/mmol, Amersham Biosciences, Inc., Uppsala, Sweden) and
linearized
of linearized pRI7 plasmid, which contains the rat insulin cDNA (18), or
in duplicates against an external standard curve with known quantities
-CCACCAATCCACACAGAGTACTTG. Unknown samples were run
50,000 insulin mRNA molecules and
50,000 pmole insulin mRNA to be measured.

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real-time PCR, insulin mRNA contents of
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Control of Insulin mRNA Stability by the 3'-UTR

RESULTS

Insulin mRNA Is Highly Abundant in Rat β-Cells—Usingeal-time PCR, insulin mRNA contents of
in vitro cultured rat pancreatic islets were quantified. We first assessed basal and
non-stimulated insulin mRNA levels by preculturing the islets
for 24 h at a substimulatory glucose concentration and in the
absence of serum. At these basal conditions, we observed that
insulin mRNA is 60-80 times more frequent than the

Lightcycle real-time PCR instrument, using the Faststart DNA Master
suggests that the vast majority of the islet population is
composed of insulin-producing cells.

For the primer design, we used the public-domain software
Primer3 (Roche Molecular Biochemicals, Mannheim, Germany).

The following primers were used: insulin forward, 5'-ACACGC-
ACCTTTGTGGTCC; insulin reverse, 5'-GGACTCGTGGCAGTGT-
TC; β-actin forward, 5'-GCCCTGGCTCCTAGCACC; β-actin reverse,
5'-CCACCAATCCACAGAGTACTTG. Unknown samples were run in
duplicates against an external standard curve with known quantities
of linearized pRI7 plasmid, which contains the rat insulin cDNA (18), or
linearized β-actin cDNA plasmid. The amplification efficiency was sim-
ilar for the reverse-transcribed islet RNA samples and pRI7 (Fig. 2A).
β-Cell number per islet was determined using flow cytometry.
Briefly, islets were trypsinized for 5 min at 37 °C to generate free islet
cells. The cells were then fixed for 5 min in 4% paraformaldehyde,
permeabilized with 1% saponin, and incubated for 20 min at room
temperature with a guinea pig anti-insulin antibody. A fluorescein
isothiocyanate-labeled anti-guinea pig antibody was added, and the
was analyzed in a FACSCalibur flow cytometer (Becton-Dickin-
son Instruments). Cells with increased FL−fluorescence were gated and
counted as β-cells.

Electromobility Shift Assay and Cross-linking Analysis—The
following RNA oligonucleotides were used: ins-PRS, 5'-CCACCAACU-
CCCAACCCUCU; mutant 1 ins-PRS, 5'-UCCCAACUCUCUCUU; mutant 2 ins-PRS, 5'-UCCCAACUCUCUCUU; mutant 3 ins-PRS, 5'-UCCCAACACACCCUU; mutant 4 ins-PRS, 5'-UCCCAACACACCCUU. All vectors were control-sequenced
by reducing SDS-PAGE and transferred to a membrane and renatured
overnight at 4°C. The membrane was then incubated in hybridization
buffer (renaturing buffer + 20 mg/ml RNA, 1 mg/ml heparin, and 0.5
mmol of 32P-labeled RNA oligonucleotide) for another 2 h. The
membrane was further washed 3 × 10 min in renaturing buffer, dried, and
finally exposed to an x-ray film. The position of the radioactive band
was detected by the Amersham ECL system (Amersham Biosciences, Inc.).

ELUTION AND ANALYSIS OF GEL SLICES CONTAINING THE RNA Oligonucleotide-Protein Complex—RNA oligonucleotide-protein binding reactions
were run on a 7% acrylamide gel and electrophoresed in 0.5 × TBE (45 mM tris Borate, 10 mM EDTA). The gel was fixed, dried, and exposed to a film
overnight at ~70 °C.

B

PTB consensus sequence
Rat tyrosine hydroxylase GCC
Human tyrosine hydroxylase GCC
Rat preproenkephalin G

PCBP consensus sequence
Rat tyrosine hydroxylase GCC
Human tyrosine hydroxylase GCC
Rat preproenkephalin G

PCBP-HEBS sequence
Rat tyrosine hydroxylase GCC
Human tyrosine hydroxylase GCC
Rat preproenkephalin G

FIG. 1. A, comparison of PCBP and PTB consensus binding sequences

with pyrimidine-rich 3'-UTR segments of TH and rat insulin mRNA. Y
denotes pyrimidines and N any nucleotide. Letters in bold mark posi-
tions of transitions or transversions that differ from the PCBP and
PTB consensus binding sequences. B, comparisons of the 2 PTB
consensus binding sequences with preproinsulin mRNA pyrimidine-rich 3'-UTR
sequences located downstream of end of coding sequence and upstream
of poly(A) signal. Letters in bold mark positions of transitions that differ
from the PTB consensus binding sequence.

Electromobility Shift Assay and Cross-linking Analysis—The
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finally exposed to an x-ray film. The position of the radioactive band
was detected by the Amersham ECL system (Amersham Biosciences, Inc.).

Northwestern Hybridization—Northwestern was performed essen-
tially as described previously by others (21). Islet proteins were sepa-
rated by 12% SDS-PAGE and transferred to a nitrocellulose filter by
incubating the membrane in renaturing buffer (21) for 2 h at room

The RNA-protein binding reaction was performed essentially as de-
scribed previously (19). To half the reactions 11 mM dithiothreitol (DTT)
was added, before addition of the probe. In some cases, the reaction
mixtures were divided into two aliquots, one which was cross-linked by
exposure to uv radiation (5 milliwatts/cm²) for 5 min and then analyzed
by reducing SDS-PAGE, the other was used directly for non-denaturing
gel electrophoresis. In the latter case, the samples were applied on a 7%
polyacrylamide gel and electrophoresed in 0.5 × TBE (45 mM tris Borate, 10 mM EDTA). The gel was fixed, dried, and exposed to a film
overnight at ~70 °C.

Elution and Analysis of Gel Slices Containing the RNA Oligonucle-
tide-Protein Complex—RNA oligonucleotide-protein binding reactions
were run on a non-denaturing gel, and the position of the retarded
and radiolabeled RNA oligonucleotide-protein complex was visualized by
exposing an x-ray film to the gel. The lanes were then cut in three parts:
above, at, and below the RNA-protein complex position. Proteins were
eluted from the gel slices in 5 mM Tris acetate, pH 8.0, 0.1% SDS, and
0.1 mM EDTA under agitation overnight. The eluates were then con-
centrated on Centricon microconcentrators (Amicon, Beverly, MA), and
the proteins were separated on a 12% SDS-PAGE and electrophoresed to
a nitrocellulose filter. The filters were hybridized with the monoclonal
anti-PTB 3 antibody (20). Horseradish peroxidase-conjugated anti-
mouse antibody (1:1000) was used as secondary antibody, which was
detected by the Amersham ECL system (Amersham Biosciences, Inc.).

The cells from each of the four dishes were then exposed for 24 h to 2.8
mg/ml actinomycin D. The cells were analyzed in a FACSCalibur flow
cytometer. The cell number per islet was determined using flow

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A

Fig. 2. Real-time PCR quantification of insulin mRNA contents. A, amplification efficiency of linearized pRI7 plasmid insulin cDNA and rat islet insulin cDNA samples in real-time PCR reactions. Series of 10-fold dilutions were analyzed in duplicates. B, precultured rat pancreatic islets were maintained for 24 h at 1.7 mM glucose and in the absence of serum. The islets, in groups of 20–30, were then stimulated for 60 min with 17 mM glucose or 5 μg/ml insulin. Insulin mRNA, β-actin mRNA, and β-cell number were quantified as described under “Experimental Procedures.” Results are means ± S.E. for six independent observations. C, precultured rat islets were maintained for 24 h at 1.7 mM glucose and in the absence of serum. The islets were then stimulated for 24 h with 17 mM glucose or with 10% Fetal Clone II. messenger for β-actin (Fig. 2B). In line with this, a short term (1 h) stimulation with a high glucose concentration did not increase the contents of insulin mRNA (Fig. 2B). Insulin has recently been reported to potently stimulate insulin gene transcription (8–10). However, addition of 5 μg/ml insulin did not affect insulin mRNA contents (Fig. 2B). These insulin mRNA estimates are based upon the assumption that the yield and efficiency of the RNA extraction and cDNA synthesis procedures are 100%. Our results might therefore be underestimates.

Next, we quantified insulin mRNA contents in response to a long term (24 h) glucose and serum stimulation. The insulin mRNA contents of control islets dropped to −20,000/β-cell (Fig. 2C). This is not surprising, because prolonged culture without glucose and serum (24 h + 24 h) is known to result in β-cell apoptosis (23). The 24-h high glucose stimulation increased insulin mRNA contents to −100,000/β-cell (Fig. 2C). Also the ratio insulin mRNA to β-actin mRNA was markedly increased (Fig. 2C).

Having established that insulin mRNA is highly abundant and only slowly increased in response to glucose, we next addressed the question whether transcriptional or post-transcriptional mechanisms mediate the regulatory effect of glucose. For this purpose, islets were precultured in the presence of serum and high glucose to bring the insulin mRNA contents up to high levels. Next, some of the islets were transferred to a low glucose-containing medium with or without actinomycin D. As expected, a 16-h incubation at a low glucose concentration promoted a small (35%) decrease in insulin mRNA (Fig. 2D). Interestingly, inhibition of RNA synthesis did not decrease insulin mRNA contents and the drop induced by low glucose was of the same magnitude in the presence as in the absence of actinomycin D (Fig. 2D). This correlates well with previous findings showing that actinomycin D does not markedly reduce insulin mRNA levels (5). This is possibly explained by inhibition of synthesis of insulin mRNA degrading factors, which would mask the inhibiting effect on insulin gene transcription (5). On the other hand, β-actin mRNA did not decrease in response to low glucose and the actinomycin D-induced decrease was more pronounced than that of insulin mRNA (Fig. 2D). Consequently, the low glucose-induced decrease in the insulin mRNA/β-actin mRNA ratio was of the same relative magnitude both with and without actinomycin D (Fig. 2D). These findings are in line with the previously established insulin mRNA half-life of 29 h at a low glucose concentration, 77 h at a high glucose concentration, and a β-actin mRNA half-life of 9 h (5, 24).

A 55–60-kDa Protein Binds to the Ins-PRS—The findings that glucose-induced changes in insulin mRNA stability control insulin mRNA contents prompted us to next investigate the mechanisms that regulate insulin mRNA stability. Probing for specific binding to the pyrimidine-rich insulin mRNA 3′-UTR sequence (ins-PRS), we observed binding of a 25-bp ins-PRS RNA oligonucleotide to a cytosolic protein, both in liver and islet cells. This binding resulted in a cross-linked complex with the combined molecular mass of 65–70 kDa (Fig. 3, A–C). If it were assumed that the RNA oligonucleotide increases the molecular mass of the complex with 10 kDa, the molecular mass of the RNA-binding protein would be 55–60 kDa. This binding

Results are means ± S.E. for six independent observations. D, precultured rat islets were maintained for 24 h at 17 mM glucose in the presence of serum. The islets were then transferred to 1.7 mM glucose with or without the presence of 5 μg/ml actinomycin D for 16 h. Results are means ± S.E. for eight observations. * denotes p < 0.05 using Student’s paired t test. + denotes p < 0.05 using one-way analysis of variance and Bonferroni’s test.
was not observed in the absence of UV cross-linking, nor when a mutated RNA oligonucleotide, in which four centrally located C were replaced by A (ins-PRS mutant 3), was used (Fig. 3A).

In liver cells, there was also prominent binding to the ins-PRS RNA oligonucleotide by a 40-kDa protein (Fig. 3A). The identity of this protein is not known, but the MW corresponds well to PCBP, which has been reported to bind to the pyrimidine-rich sequence of TH mRNA (25). Binding of the 40-kDa protein to the ins-PRS RNA oligonucleotide was, however, not consistently observed in islet cells (Fig. 3B and C).

The Binding Activity of the 55–60-kDa Protein to Ins-PRS Is Stimulated by Reducing Agents and Glucose—Using cytosolic extracts from non-stimulated rat pancreatic islets, we observed that the reducing agents DTT (Fig. 3B) or β-mercaptoethanol (data not shown) increased binding of the 55–60-kDa protein to ins-PRS. The enhancing effect of DTT was dose-dependent and reached maximum at 5–10 mM (data not shown). The increased binding of the 55–60-kDa protein to ins-PRS in response to DTT was also observed when binding reactions were analyzed by non-denaturing gel electrophoresis (Fig. 3D). In this case, binding was visualized by retardation of the radioactive RNA oligonucleotide, and the intensity of the retarded band corresponded well to the intensity of the 65–70-kDa protein-RNA oligonucleotide complex observed in parallel cross-linking experiments (Fig. 3C and D). The effect of reducing agents was not as clear in islet homogenates stimulated with a high glucose concentration as compared with non-stimulated islets (Fig. 3C and D). Instead, the ins-PRS binding activity of glucose-stimulated islets was enhanced in the absence of DTT and not further increased in the presence of DTT. These findings suggest that the ins-PRS binding activity is maximally stimulated in the presence of DTT and that differences observed in binding activity in the absence of DTT may reflect the in vivo activity of the 55–60-kDa protein. Thus, by expressing...
ins-PRS binding activity as the ratio binding activity –DTT/ +DTT, we correct for differences in total amount of the 55–60-kDa protein present in islet samples. When analyzing the effect of glucose, it was found that the ins-PRS binding activity in rat islet cytosol was significantly increased by 28 mM, but not by 5 mM glucose (Fig. 4). In nuclear extracts, both 5 and 28 mM glucose increased ins-PRS binding activity as compared with no glucose (Fig. 4). Thus, glucose-induced stabilization of insulin mRNA is paralleled by increased binding of the 55–60-kDa protein to ins-PRS.

Also human insulin mRNA 3′-UTR contains a pyrimidine-rich motif (Fig. 1B). To determine whether binding in human islet cytosol extracts to the human ins-PRS occurs, we analyzed binding reactions by non-denaturing gel electrophoresis. It was observed that a similar gel retardation product was present in human islet extracts as in rat islet extracts (Fig. 3E). This indicates that a similar insulin mRNA 3′-UTR-protein complex is formed in human islets as in rat islets.

The 55–60-kDa Ins-PRS Binding Protein Is Probably PTB—To determine the identity of the ins-PRS-binding protein, we performed supershift analysis using antibodies specific for hnRNP-K, PCBP, and PTB. The antibodies against hnRNP-K and PCBP did not generate a supershift (Fig. 5A, results not shown). However, two different PTB monoclonal antibodies specifically abolished the ins-PRS/protein complex, an effect that was not observed with control asacites fluid (Fig. 5A). This indicates that antibody binding to PTB blocks PTB binding to the ins-PRS RNA oligonucleotide.

The Northwestern technique was utilized to probe for ins-PRS binding activity of proteins immobilized to a nitrocellulose filter. We observed ins-PRS binding activity of a renatured protein, which migrated to the same position as PTB (Fig. 5B).

Further evidence in support for PTB as the ins-PRS-binding protein was obtained by analyzing the presence of PTB at the position of the ins-PRS-protein complex in a non-denaturing electrophoresis shift assay gel. By eluting proteins from above, at and below the position of the ins-PRS-protein complex, we observed PTB immunoreactivity at the complex position in samples with ins-PRS probe present during the binding reaction, but not in samples without (Fig. 5C). The remaining PTB immunoreactivity was located above and not below the position of the ins-PRS-protein complex. These results indicate that the fraction of PTB that binds the ins-PRS RNA oligonucleotide alters its three-dimensional conformation so that it migrates faster in a non-denaturing gel.

PTB and Ins-PRS Stability Is Not Affected by DTT or Glucose—To exclude the possibility that increased binding of PTB to ins-PRS is due to altered stability in response to DTT or glucose, islet cell homogenates were incubated for 50 min in homogenization buffer with or without 11 mM DTT. Samples were then analyzed by immunoblotting using the anti-PTB-3 antibody and counterstained with Amido Black. It was observed that the in vitro stability of PTB (Fig. 6A) and ins-PRS (Fig. 6B) was not affected by the addition of DTT. In addition, islets were incubated for 1 h in medium containing 2.8 or 28 mM glucose, and the amount of expressed PTB was examined by immunoblotting. We could not observe any differences in PTB expression between the two groups (Fig. 6C), indicating that a 1-h incubation period is not sufficient to alter PTB contents.

Inhibition of PTB Binding to Ins-PRS Results in mRNA Destabilization—Mutation of the critical pyrimidines to purines resulted in abolished binding of PTB to ins-PRS in vitro (Fig. 3A). To assess whether mRNA stability is affected by PTB
binding to ins-PRS in vivo, we lipofected dispersed rat islet cells with pCR TM-CAT vector with or without wild-type or mutated ins-PRS. In the presence of actinomycin D, we observed that the reporter gene mRNA containing the wild-type rat insulin I ins-PRS was equally abundant as the mRNA lacking ins-PRS (Fig. 7A). However, a mutation of one of the pyrimidines to a purine in the PTB core-binding site resulted in a marked destabilization of the mRNA, whereas a mutation of a purine to a pyrimidine outside the core-binding site had no effect (Fig. 7A). Similar results were obtained in cells incubated in the absence of actinomycin D (Fig. 7B), indicating that transcription of the reporter gene was not affected by the ins-PRS mutation. In cells expressing wild-type ins-PRS, there was no increase in reporter gene mRNA levels in response to glucose.

DISCUSSION

To our knowledge, this is the first study to quantify insulin mRNA in isolated pancreatic islets. At physiological conditions, i.e. in the presence of serum, the β-cell content of insulin mRNAs was at least 40,000–100,000 molecules, depending upon the glucose concentration of the culture medium. This finding is in agreement with the early and indirect assessment (6), which indicated a β-cell insulin mRNA content of 50,000–150,000 molecules. A typical mammalian cell contains ~360,000 mRNAs in its cytoplasm (26). Assuming that the same number applies to the β-cell, insulin mRNA would constitute up to 30% of all mRNA, which correlates well with the percentage of insulin protein synthesized (1).

We did not observe any increase in insulin mRNA in response to a short term (60 min) glucose stimulation. This is, however, not surprising considering that insulin mRNA is vastly abundant in β-cells. The transcriptional output of insulin mRNA by the two insulin genes present in rat has not been determined experimentally. However, a theoretical calculation of the maximal insulin mRNA synthesis rate tells us that no more than 2500 transcripts/h are produced. This assessment is based upon an elongation rate of 30 nucleotides per second and that 10 RNA polymerases transcribe each insulin gene simultaneously. Although this is most likely an overestimate, it is far from the net increase of 40000 molecules, which is the minimum number of molecules necessary to double the insulin mRNA content of low glucose cultured islets in 1 h. In our
hands, the insulin mRNA content is only slightly decreased by a 16 h glucose withdrawal. The same decrease was observed in islets with inhibited RNA polymerase activity. This indicates that control of insulin mRNA stability contributes significantly to the regulation of insulin mRNA levels. Indeed, it is generally agreed that the contents of abundant and long lived messengers are subject to post-transcriptional, rather than transcriptional control. This view is challenged by Leibiger and co-workers (8–10), who report that insulin mRNA contents are increased up to 5-fold in response to a 60-min glucose stimulation. The reason for the apparent incompatibilities between the present work and the work by the Leibiger group is unclear.

Although glucose is known to specifically increase insulin mRNA stability (5), little is hitherto known of the molecular mechanisms underlying this event. However, a recent report identified the 3'-UTR of insulin mRNA as critical for control of messenger stability (11). The UUAGA sequence, located between the polyadenylation signal and the polyadenylation site, was suggested to be important because of its conservancy (11). Alternatively, a closer analysis of the 3'-UTR of rat insulin mRNA (Fig. 1A) shows that the region just up-stream of the polyadenylation signal and downstream of the termination codon contains a pyrimidine-rich sequence, with similarities to the 3'-UTR of insulin mRNA as critical for control of messenger stability (11). The UUAGA sequence, located between the polyadenylation signal and the polyadenylation site, was suggested to be important because of its conservancy (11).

In summary, we have identified an interaction between the ins-PRS-binding protein and that this event is evolutionarily conserved.

Species with no ins-PRS are frog, hagfish, and chimpanzee. The frog and particularly the hagfish are evolutionary distant species that both have insulin mRNA 3'-UTRs that are several hundred bases long, suggesting that these mRNAs utilize other mechanisms for messenger stability control. The chimpanzee, however, has lost 48 bp of its insulin gene due to a deletion at a site just after the reading frame (30). This results in a very short 3'-UTR without any pyrimidine-rich motif. Had it been possible to perform experimental studies with chimpanzee islets, it would have been very interesting to determine whether insulin mRNA levels in the chimpanzee are governed by the same signals as in rodent and human islets. Type 2 diabetes mellitus has been observed in the chimpanzee and may be more frequent than expected (31).

The main known function of PTB is to inhibit mRNA splicing (32). In addition, PTB is thought to play an important role in events such as cap-independent translation (33), RNA polyadenylation, (34) and RNA localization (35). The PTB-binding site in insulin mRNA is located just upstream of the polyadenylation signal, and it is not unlikely that PTB, in addition to regulation of mRNA stability, is involved in polyadenylation, transport, and/or translation. Moreover, PTB exists in three different isoforms, with differential alternative splicing activity, and PTB homologues with cell type-specific expression patterns have recently been identified (36). Consequently, the possibility exists that PTB isoforms and the different PTB homologues have overlapping but distinct RNA binding specificities and that a specific PTB isoform or homologue is expressed in β-cells with the purpose to modulate insulin mRNA levels.

Although PTB mRNA expression is up-regulated about five times in the β-cell line MIN6, in response to a 24-h glucose stimulation (15), we could not detect any differences in protein expression after a 1-h incubation at different glucose concentrations. This suggests that acute alterations in the PTB binding activity is regulated by post-translational modifications rather than by increased PTB gene expression. Furthermore, PTB is known to interact with other members of the hnRNP family such as hnRNP-E2 (PCBP 2), -K, and -L, which are all expressed in islets (Refs. 33 and 37, present results). Thus, the interaction of PTB with additional RNA-binding proteins may be necessary events in glucose-mediated regulation of insulin mRNA stability.

In summary, we have identified an interaction between the rat insulin I 3'-UTR and the protein PTB, an event that may be necessary for glucose-induced stabilization of insulin mRNA.

Acknowledgments—The excellent technical assistance of Ing-Marie Morsare and Ing-Britt Hallgren is gratefully acknowledged.
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Control of Insulin mRNA Stability in Rat Pancreatic Islets: REGULATORY ROLE OF A 3′-UNTRANSLATED REGION PYRIMIDINE-RICH SEQUENCE
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J. Biol. Chem. 2002, 277:1099-1106.
doi: 10.1074/jbc.M108340200 originally published online November 5, 2001

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