Original article

MOLECULAR METABOLISM

PTBP1 is required for glucose-stimulated cap-independent translation of insulin granule proteins and Coxsackieviruses in beta cells

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ABSTRACT

Glucose and GLP-1 stimulate not only insulin secretion, but also the post-transcriptional induction of insulin granule biogenesis. This process involves the nucleocytoplasmic translocation of the RNA binding protein PTBP1. Binding of PTBP1 to the 3′-UTRs of mRNAs for insulin and other cargoes of beta cell granules increases their stability. Here we show that glucose enhances also the binding of PTBP1 to the 5′-UTRs of these transcripts, which display IRES activity, and their translation exclusively in a cap-independent fashion. Accordingly, glucose-induced biosynthesis of granule cargoes was unaffected by pharmacological, genetic or Coxsackievirus-mediated inhibition of cap-dependent translation. Infection with Coxsackieviruses, which also depend on PTBP1 for their own cap-independent translation, reduced instead granule stores and insulin release. These findings provide insight into the mechanism for glucose-induction of insulin granule production and on how Coxsackieviruses, which have been implicated in the pathogenesis of type 1 diabetes, can foster beta cell failure.

Keywords Beta cells; Diabetes; Insulin; Polypyrimidine tract-binding protein; Secretory granules; Translation; Virus

1. INTRODUCTION

Hyperglycemia and incretins prompt pancreatic beta cells to produce and release insulin. Rapid induction of insulin granule biogenesis to replenish the hormone stores is of physiological relevance given the preferential release of newly synthetized insulin [1—3]. This process is not transcriptionally regulated, but depends on post-transcriptional mechanisms involving polypyrimidine tract-binding protein 1 (PTBP1) [4,5]. PTBP1 binds to polypyrimidine-rich sequences [6] of single-stranded RNAs and has been implicated in alternative splicing [6,7] and polyadenylation of pre-mRNAs [8] as well as stability [4,5] and translation initiation [9] of mRNAs.

Stimulation of insulinoma and primary beta cells with glucose and GLP-1 induces PTBP1 translocation from the nucleus to the cytosol [5,10,11]. Binding of cytosolic PTBP1 to the 3′-untranslated regions (UTRs) of mRNAs for insulin and other insulin granule proteins enhances their stability and translation, and thereby granule biogenesis, while silencing of PTBP1 in insulinoma cells inhibits glucose- and incretin-stimulated insulin secretion [4,5,10,12], Notably, the impaired ability of diabetic islets isolated from partially pancreatectomized subjects to rapidly upregulate total insulin levels in response to glucose-stimulation correlates with increased nuclear retention of PTBP1 [11]. Furthermore, PTBP1 has been identified as a novel risk gene for type 2 diabetes associated with reduced insulin secretion [13]. Hence, the study of PTBP1 function in beta cells is relevant for type 2 diabetes.

PTBP1 is a key internal ribosomal entry site (IRES)-trans-acting factor (ITAF) for cap-independent translation of various positive single-stranded RNA viruses [7,14—16], including human Enteroviruses (EVs). In the case of cap-dependent translation interaction of eIF4G/A complex with the cap-binding protein eIF4E results in the recruitment of the 40S ribosomal subunit to the very 5′-terminus of m^7GpppN-capped mRNAs. Cap-independent translation involves instead the recruitment of the 40S ribosomal subunit to *cis*-acting IRES located within the 5′-UTR of RNAs, in closer proximity to the start codon for translation [15,17,18]. EVs inhibit translation of the host cell while exploiting its translational machinery for cap-independent translation of their uncapped RNA genome [19,20]. Intriguingly, EVs such as Coxsackieviruses are regarded among the environmental factors that may trigger or accentuate loss of immune self-tolerance towards beta cells in type 1 diabetes (T1D) [21—24].

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Abbreviations: CV, Coxsackievirus; eIF4E-V5, eIF4E tagged at its C-terminus with a V5-epitope; ER, endoplasmic reticulum; EV, Enterovirus; F, Faulkner; FL, firefly luciferase; IRES, internal ribosomal entry site; ITAF, IRES-trans-acting factor; mTORC1, mammalian Target Of Rapamycin Complex 1; MCA, MIN6 cell adapted; PABP, poly(A)-binding protein; PC, prohormone convertase; PTBP1, polypyrimidine tract-binding protein 1; S6K1, p70S6 Kinase 1; T1D, type 1 diabetes; UTR, untranslated region

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In this study we sought to investigate whether glucose-dependent, PTBP1-regulated translation of mRNAs for insulin granule proteins is cap-independent and whether this process is affected upon infection of rodent insulinoma INS-1 and MIN6 cells as well as mouse pancreatic islets with Coxsackieviruses.

2. MATERIAL AND METHODS

2.1. Material

The following commercial antibodies were employed: mouse monoclonal antibodies anti-PTBP1, anti-V5 (Invitrogen), anti-m3G/m7G-cap (Synaptic System), anti-γ-tubulin and anti-insulin (for western blotting) (Sigma), rabbit polyclonal antibodies anti-eIF4E, anti-ph-eIF4E (Ser-209), anti-4E-BP, anti-eIF4G, anti-AKT, anti-ph-AKT (Ser-473), anti-p70S6K, anti-ph-p70S6K (Thr-389) and anti-PABP (Cell Signaling Technology), anti-CgA (Abcam), anti-PC1/3 and anti-PC2 (GeneTex), guinea pig antibody anti-insulin (for immunocytochemistry) (Abcam), goat anti-mouse, anti-rabbit and anti-guinea pig IgGs conjugated with Alexa 488 or Alexa 568 (Molecular Probes), goat anti-mouse and anti-rabbit IgGs conjugated to horseradish peroxidase (Bio-Rad). The mouse anti-ICA512 mAb has been previously described in Ref. [25]. The following reagents were from commercial sources: Rapamycin and LY294002 (Cell Signaling Technology), the eIF4E/eIF4G interaction inhibitor (Calbiochem), m^7GpppG cap-analog (New England Biolabs), m^7GTP-Sepharose (GE Healthcare) and Dynabeads M270 streptavidin (Invitrogen), reagents for luciferase assay (Promega), ^{35}S-methionine (Hartmann-Analytics), pro-/insulin ELISA (Mercodia) and insulin RIA (Millipore).

2.2. Islet isolation and cell culture

Pancreatic islets were isolated from C57BL/6JRj mice by collagenase digestion and density gradient centrifugation as described previously in Ref. [26]. Mouse insulinoma MIN6 and rat insulinoma INS-1 cells were kind gifts from Jun-ichi Miyazaki (Osaka, Japan) and Claes Wollheim (Geneve, Switzerland), respectively, and were cultured as described in Refs. [5,27]. Cells were kept in resting medium (15 mM HEPES, pH 7.4, 5 mM KCl, 120 mM NaCl, 24 mM $NaHCO_3$, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 3.3 mM glucose, 1 mg/ml ovalbumin) for 1 h before stimulation for 2 h by addition of glucose as indicated. All inhibitors were added at the indicated concentrations to the resting and stimulating media. Two hours after stimulation with glucose cells or islets were harvested. *De novo* protein synthesis was validated by metabolic labeling of MIN6 cells with 100 μCi ^{35}S-methionine/35 mm well for 2 h. After 5 washes the cells were extracted in lysis buffer containing 1% Triton X-100. After normalization of the protein concentrations the immunoprecipitations was carried out following standard protocols [28]. Biosynthesis of insulin was investigated as described in Ref. [29].

2.3. Cloning

The 5′-UTRs of rat *PC1/3*, *PC2*, *insulin1* and *2* were amplified by RT-PCR from INS-1 cell total RNA and cloned into pGL3-B (Promega). The 5′-UTR of rat *ICA512* mRNA was obtained by 5′-RACE based on the public sequence NM_053881. Mutation of polypyrimidine tracts in rat *ICA512* and *PC2* mRNA 5′-UTRs was carried out with the QuikChange Site-Directed Mutagenesis Kit (Stratagene). For overexpression of eIF4E the coding sequence was cloned into pcDNA3.1 (Invitrogen) using the Directional cloning kit.

2.4. Transfection

DNA was transiently transfected into MIN6 or INS-1 cells using an AMAXA electroporator, as described in Ref. [5]. siRNAs were transfected into MIN6 cells with Dharmafect 4 solution (Thermo Scientific). Longer RNA molecules as well as the m^7GpppG cap-analog were transfected using Lipofectamin 2000 (Invitrogen).

2.5. Virus infection

CVB5 F or MCA were propagated in GMK cells as described in Ref. [30]. For infection, 5×10^5 MIN6 cells were incubated with 2.25×10^5 $TCID_{50}$ CVB5 F or MCA/5×10^5 cells as described in Ref. [30].

2.6. Protein analysis

Cells and purified islets were extracted in lysis buffer [20 mM TRIS/HCl, pH 8.0, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1% protease inhibitor cocktail (Sigma) and 1% phosphatase inhibitor cocktail (Calbiochem)]. Protein concentration in the detergent soluble material was measured by BCA assay (Pierce). Cell extracts were separated by SDS-PAGE and immunoblotted as described in Ref. [5]. Chemiluminescence was performed using the Supersignal West Pico Substrate (Pierce) and detected with a LAS 3000 Bioimaging System (Fuji). Total protein synthesis was validated by metabolic labeling of insulinoma cells with 100 μCi ^{35}S-methionine/35 mm well for 2 h. After 5 washes the cells were extracted and the protein precipitated with 10% TCA.

2.7. Immunocytochemistry

MIN6 cells were grown on cover slides, fixed after treatments with 3% paraformaldehyde and permeabilized with 0.2% saponin. Immunostaining, image acquisition and processing were performed as described in Ref. [5].

2.8. RNA interference

Short interfering double-stranded RNA (siRNA) oligonucleotides for mouse *eIF4E* (NCBI accession number NM_007917) were synthesized with the Silencer siRNA Construction Kit (Ambion) using the following primers: *eIF4E* sense primer 1, 5′-aaccttcgattgatctctaagc*cctgtctc*; *eIF4E* antisense primer 1, 5′-aacttagagatcaatcgaagg*cctgtctc*; *eIF4E* sense primer 2, 5′-aaggtgataagatagcaatat*cctgtctc*; *eIF4E* antisense primer 2, 5′-aaatattgctatcttatcacc*cctgtctc*; *eIF4E* sense primer 3, 5′-aagtctcattcgcctttgtct*cctgtctc*; *eIF4E* antisense primer 3, 5′-aaagacaaaggcgaatgagac*cctgtctc*. Control scrambled siRNA oligonucleotides were previously described in Ref. [5]. siRNA oligos for mouse *PTBP1* (NCBI accession number NM_008956) and the corresponding control siRNA were obtained from RIBOXX.

2.9. Real-time PCR

Total RNA from INS-1 cells was prepared with the RNeasy Kit (QIAGEN). 1 μg total RNA was reverse transcribed with SuperScript II reverse transcriptase (Invitrogen) and oligo d(T) primer. mRNA levels were measured by quantitative real-time PCR with the qPCR GoTaq Kit (Promega) and a MX4000 Thermocycler (Stratagene). Normalization of real-time PCR data was performed by parallel amplification of rat β-actin mRNA. The used primers have been previously described in Ref. [10].

2.10. Luciferase assays

INS-1 cells were co-transfected with firefly and renilla luciferase constructs. The firefly luciferase activity was measured 4 days after transfection and normalized versus that of renilla luciferase. Luciferase activity in MIN6 cells 1 day after transfection of reporter RNA was measured as for INS-1 cells.

2.11. In vitro RNA binding assay

The mRNA 5′-UTRs of *ICA512*, *PC1/3*, *PC2*, *insulin1* and *2* as well as CVB5 MCA RNA were biotinylated by T7 in vitro transcription (Biozym).

