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Novel splice variant of mouse insulin2 mRNA: Implications for insulin expression

Amaresh C. Panda, Shardul D. Kulkarni, Bhavana Muralidharan, Baskar Bakthavachalu, Vasudevan Seshadri *

National Centre for Cell Science, Ganeshkhind, Pune 411 007, India

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ABSTRACT

Insulin is a secreted peptide that controls glucose homeostasis in mammals, and insulin biosynthesis is regulated by glucose at many levels. Rodent insulin is encoded by two non-allelic genes. We have identified a novel splice variant of the insulin2 gene in mice that constitutes about 75% of total insulin2 mRNA. The alternate splicing does not alter the ORF but reduces the 5′UTR by 12 bases. A reporter gene containing the novel short 5′UTR, is more efficiently expressed in cells, suggesting that alternative splicing of insulin mRNA in mice could result in an additional level of regulation in insulin biosynthesis.
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1. Introduction

Insulin, a pancreatic peptide hormone is regulated by glucose at various steps of insulin gene expression such as transcription, splicing, mRNA stability and translation [1–4]. The increase in insulin production within an hour of the glucose treatment occurs primarily due to increase in translation with no significant enhancement in mRNA levels [5]. This upregulation is mediated by binding of specific proteins in a sequence and structure specific manner to the un-translated regions (UTR) of insulin mRNA [6,7]. Thus, any alteration to the 5′UTR has the potential for differential regulation of insulin gene expression.

Mouse insulin is encoded by two non-allelic genes [8]. The insulin2 gene has two introns while the insulin1 gene contains only one intron and is believed to have evolved by retrotransposon mediated gene duplication with loss of intron2 [9]. Both the introns of insulin2 are flanked by characteristic GU-AG sequence. Intron1 is spliced inefficiently as compared to the intron2, as a result about 2–10% of the cytoplasmic insulin2 mRNA contain intron1 [3]. This intron1 containing RNA has increased translation efficiency and its level is increased in diabetic/insulin resistant mouse models [10]. We hypothesized that due to the inefficient splicing, intron1 can be alternatively spliced by using one or more cryptic splice-sites that are present in the vicinity. Analysis of the mouse insulin2 gene sequences identified the presence of a putative alternative 3′-splice-site for intron1. This new cryptic splice-site is used predominantly and the alternatively spliced mRNA is expressed in multiple mouse species as well as in insulin producing cell lines. We also show that reporter mRNA containing the new splice variant at the 5′UTR is expressed more efficiently when compared to the splice variant of the insulin2 mRNA.

2. Materials and methods

2.1. Mouse islet isolation

All animal work was performed as per the IEAUC guidelines and approval. Six to eight weeks old BALB/c or C57BL6 mice were used. Pancreatic islets were prepared as described previously [11] and purified on Ficoll gradient.

2.2. RNA extraction, RT-PCR, and structure prediction

Total RNA was prepared using TRIzol reagent and cDNA was synthesised using ImProm-II Reverse Transcriptase Kit (Promega). For sequencing of splice variants, Insulin2 gene was PCR amplified with gene specific primers 1 and 4 (Table S1). Secondary structure of the RNA was predicted using the m-fold algorithm [12].

2.3. Cell culture and transfection

2×10^5 cells (βTC6 and HEK293), maintained in DMEM, 10% FCS, 100 U/ml penicillin and streptomycin, were transfected with 5′UTR containing firefly luciferase (100 ng) and Renilla luciferase (1 ng) DNA using Lipofectamin 2000 (Invitrogen). Cells were harvested after 16 h and the Firefly and Renilla luciferase activities were measured using the DLR Kit (Promega).

* Corresponding author. Fax: +91 20 25692259.
E-mail addresses: seshadriv@nccs.res.in, seshadrv@yahoo.com (V. Seshadri).

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Table 1
Comparison of the splice-sites of intron1 of insulin.

Genes	5′-Splice-site of intron1	3′-Splice-site of intron1	Intron1 (bases)	Intron2 (bases)
Consensus	AG/GURAG	YAG/GU		
Human insulin	AG/GUCUG	CAG/AU	179	786
Rat Ins 1	AG/GUAUG	CAG/GU	119	0
Rat Ins 2	AG/GUAUG	CAG/GU	119	499
Mouse-Ins1	AG/GUAUG	CAG/GU	118	0
Mouse-Ins2	AG/GUACU	CAG/CC	102	486
Mouse-Ins2-S	AG/GUACU	CAG/GU	114	486

2.4. Polysome fractionation

The Polysome fractionation was done as described previously [13]. Briefly, 5×10^6 βTC6 cells were washed and homogenized in 1.5 ml of the homogenization buffer (10 mM Tris-pH 8.0, 25 mM KCl, 10 mM MgCl2, 250 mM sucrose, 2 mM DTT, 0.1 μg/ml cycloheximide, protease inhibitor, 100 U/ml RNasin). After an initial 10 s centrifugation at 12 000×*g* to remove the nuclei, the supernatant was centrifuged for 12 min at 130 000×*g*. RNA was isolated from the pellet (polysome) and the supernatant (non-polysome) fraction.

2.5. Plasmid constructs

Double stranded oligonucleotides corresponding to mIns2, mIns2-S 5′UTR and mIns2 3′UTR were cloned in pSP64-luc-Cp3′UTR-polyA plasmid (gift from Dr. Paul Fox, LRI, Cleveland Clinic Foundation). Insulin 5′-UTR-Luc-3′UTR fragments from the pSP64 vector were sub cloned in pcDNA3 vector for expression in eukaryotic cell lines.

2.6. RNA-EMSA

The cytoplasmic extracts were prepared as described previously [7]. RNA-EMSA was done with 2 μg of extracts. For competition experiments, 200 ng (∼70-fold molar excess) of unlabeled RNA was added to the reaction mix and incubated at room temperature for 5 min, prior to the addition of extract.

3. Results

3.1. A novel insulin splice variant is expressed in mouse pancreatic islets

Introns of the mouse insulin2 RNA splice out with different efficiency. The 5′ (AG/GU) as well as the 3′-splice-site (AG/CC) sequence of intron1 are not optimal for mammalian splicing (Table. 1). Sequence analysis of insulin2, revealed the presence of a potential 3′-splice-site, 12 bp downstream to the known splice-site (Fig. 1A). RT-PCR amplification of the insulin mRNA from islets of BALB/c mice using primers in the exon1 and exon2 resulted in two specific products corresponding to the expected size of the two alternately spliced mRNA (Fig. 1B). Sequence analysis as well as restriction digestion with specific restriction enzyme (BstNI) confirmed that the shorter product (mIns2-S) corresponds to the splice variant that uses the cryptic splice-site and the longer

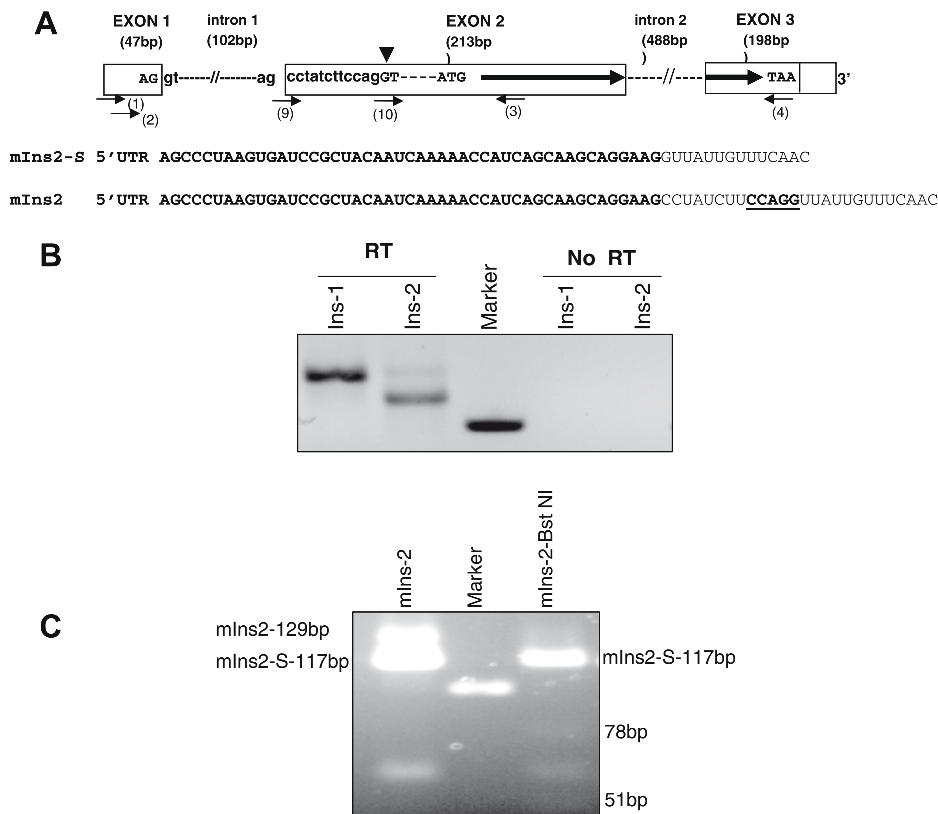

Fig. 1. Mouse Insulin2 splice variant in pancreatic islets. (A) Schematic representation of the mouse proinsulin2 gene. Insulin2 gene consists of three exons (boxes), and two introns, (dotted lines). The translation start site is in exon 2 and the stop codon in exon 3. The 5′UTR includes exon 1 and part of exon 2; the arrow head shows the new 3′-splice-site junction. The position of the primers used for RT-PCR is indicated with numbers. (B) Mouse islet cDNA was used for PCR of insulin1 with primers 5 and 6, and insulin2 with the primers 2 and 3. PCR reactions were resolved on agarose gel and samples are as indicated. (C) Deletion of 12 bases in the mIns2-S isoforms remove the *Bst*NI recognition sequence (underlined in A), hence only the mIns2 isoform will be digested and 78 bp and 51 bp fragments will be released.

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