Biochemical and Biophysical Research Communications 401 (2010) 203–206

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The role of protein kinase CK2 in the regulation of the insulin production of pancreatic islets

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ARTICLE INFO

Article history:
Received 2 September 2010
Available online 16 September 2010

Keywords:
Protein kinase
Insulin
Regulation
Phosphorylation
Inhibition

ABSTRACT

An appropriate regulation of the insulin production and secretion in pancreatic β-cells is necessary for the control of blood glucose homeostasis. The pancreatic duodenal homeobox factor-1 (Pdx-1) is among the various factors and signals which are implicated in the regulation of the insulin synthesis and secretion in the pancreatic β-cells. Recently, we identified Pdx-1 as a substrate for protein kinase CK2. Since CK2 is implicated in the regulation of many different cellular signaling pathways we now asked whether it might also be involved in the regulation of the insulin regulation in β-cells. Here, we show that insulin treatment of β-cells resulted in an elevated CK2 kinase activity. On the other hand down-regulation of CK2 activity by quinalizarin led to an elevated level of insulin. These results demonstrate that CK2 is implicated in the insulin regulation on pancreatic β-cells.

1. Introduction

Insulin is secreted from the β-cells of the pancreatic islets in response to an elevated blood glucose concentration. Increased glucose uptake in various organs results in a normalization of the blood glucose concentration. When this occurred, insulin secretion is switched off. Pancreatic β-cells act as glucose sensor and simultaneously integrate different signals to synthesize and secrete insulin. Glucose enters the β-cells through glucose transporter-2 (Glut-2) and is then phosphorylated by glucokinase to produce glucose-6-phosphate. Insulin, Glut-2 and glucokinase expression is regulated by a transcription factor called Pdx-1. Pdx-1 is the pancreatic duodenal homeobox factor-1, which is also known as IDX-1, STF-1 and Igf-1. The human Pdx-1 gene is located on chromosome 13q12.1 [28]. It codes for a protein with 283 amino acids. The N-terminus of Pdx-1 contains the transactivation domain. The middle region contains a homeobox domain, which is responsible for DNA binding and protein–protein interactions. Although the role of the C-terminus is poorly understood there are indications that the C-terminus is required for full transactivation function [19,22]. Pdx-1 is post-translationally modified by O-linked N-acetylglucosamine [9], sumoylation [12] and phosphorylation. Among the kinases that phosphorylate Pdx-1 is glycogen synthase kinase 3 (GSK3) [4], DNA-PK [16], Per-Arnt-Sim-kinase [2] and HIPK2 [5]. We have recently identified protein kinase CK2 as another kinase, which phosphorylates Pdx-1 at threonine 231 and serine 232 [21].

Protein kinase CK2 is a multipotential serine/threonine kinase consisting of a $\alpha_2\beta_2$ or $\alpha'_2\beta_2$ holoenzyme with the α-subunits of 43 kDa, the α′-subunits of 37 kDa and the β-subunits of 24–27 kDa (for review see [18,27,29]). A variety of proteins in the cytosol, nucleus and membranes have been identified as substrates for protein kinase CK2 [20]. There are some early indications that CK2 might be implicated in the insulin mediated signaling pathways as insulin increases CK2 activity in 3T3-L1 mouse adipocytes [26], rat hepatoma cells [26] and fibroblasts [13]. However, nothing is known about the insulin action on CK2 in pancreatic β-cells. Therefore, in the present study we analyzed the influence of insulin on CK2 activity in pancreatic β-cells. Furthermore, we studied the insulin secretion of β-cells after inhibition of CK2 activity.

2. Material and methods

2.1. Cell culture, reagents and antibodies

The mouse βTC-3 or Min6 cell lines were maintained in Dulbecco's modified Eagle's medium (Sigma) containing 5.5 mM D-glucose and 2 mM glutamine supplemented with 15% (v/v) fetal bovine serum, 100 μM β-mercaptoethanol in humidified 5% CO_2 at 37 °C. The characteristics of both cell lines are very similar to those of isolated islets, indicating that this cell line is an appropriate model for studying the mechanism of glucose-stimulated insulin secretion in pancreatic β-cells [7,11]. The CK2 inhibitor quinalizarin (Labotest OHG, Germany) [6] was dissolved in dimethyl sulfoxide (DMSO) to a 10 mM stock solution, which was used in a final concentration of 50 μM. Detection of CK2 was

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doi:10.1016/j.bbrc.2010.09.028

performed by using rabbit anti-peptide sera #26 (α-subunit), #30 (α′-subunit) and #32 (β-subunit) [8]. An anti-β-tubulin antibody was purchased from Santa Cruz Biotechnology.

2.2. Cell extractions

Cells were scraped off the plate with a rubber policeman and together with floating cells sedimented by centrifugation (7 min, 400×*g*). Cells were washed with cold phosphate buffered saline (PBS) and lysed with the double volume of RIPA buffer (50 mM Tris/HCl, pH 8.0, 150 mM NaCl, 0.5% sodium desoxycholate, 1% Triton X-100, 0.1% sodium dodecylsulfate) supplemented with the protease inhibitor cocktail complete™ according to the instructions of the manufacturer (Roche Diagnostics, Mannheim, Germany). After lysis, cell debris was removed by centrifugation. The protein content was determined according to a modified Bradford method with the BioRad reagent dye (BioRad, Germany).

2.3. CK2 in vitro kinase assay

To determine the activity of CK2 after its inhibition, cells were treated with quinalizarin or left untreated, lysed and the extracts were used in a kinase filter assay. In this assay, we measured the incorporation rate of [γ^{32}P] phosphate into the synthetic CK2 specific substrate peptide with the sequence RRRDDDSDDD [14]. Twenty microliters kinase buffer (50 mM Tris/HCl, pH 7.5, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM dithiotreitol (DTT)) containing 30 μg proteins were mixed with 30 μl CK2 mix (25 mM Tris/HCl, pH 8.5, 150 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 50 μM ATP, 0.19 mM substrate peptide) containing 10 μCi/500 μl [γ^{32}P] ATP. The mixture was spotted onto a P81 ion exchange paper. The paper was washed with 85 mM H_3PO_4 for three times. After treatment with ethanol the paper was dried and the Čerenkov-radiation was determined in a scintillation counter.

2.4. SDS–polyacrylamide gel electrophoresis and Western blot analysis

Proteins were analyzed by SDS–gel electrophoresis according to the procedure of Laemmli [15]. Proteins dissolved in SDS buffer (130 mM Tris/HCl, pH 6.8, 0.02% bromophenol blue (w/v), 10% β-mercaptoethanol, 20% glycerol (v/v), and 4% SDS) were separated on an SDS–polyacrylamide gel in electrophoresis buffer (25 mM Tris/HCl, pH 8.8 192 mM glycine, and 3.5 mM SDS) and transferred onto a PVDF Western blotting membrane (Roche Diagnostics, Mannheim, Germany) in a buffer containing 20 mM Tris/HCl, 150 mM glycine, pH 8.3. The membrane was probed by immunoblotting with appropriate diluted primary and secondary antibodies and assayed with the Lumi Light system (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions.

2.5. Determination of insulin secretion in Min6 cells treated with quinalizarin

Confluent Min6 cells were seeded until 40–50% confluence in 24-well plates. After pre-incubating with normal growth medium with or without 50 μM quinalizarin overnight, the cells were then washed twice with Krebs–Ringer bicarbonate HEPES buffer (KRBH) (125 mM NaCl, 5.9 mM KCl, 5.0 mM $NaHCO_3$, 1.2 mM $MgCl_2$, 1.3 mM $CaCl_2$, 25 mM HEPES, pH 7.4, and 4 mM glucose). Bovine serum albumin (BSA) (0.1%, w/v) was added as an insulin carrier. Next, cells were incubated for 4 h in KRBH containing 0.1% bovine serum albumin supplemented with or without 50 μM quinalizarin. The medium was replaced by fresh KRBH containing 0.1% BSA for another 20 min. Incubation was stopped on ice, and the supernatants were collected for insulin content assays using a rat/mouse insulin enzyme-linked immunosorbent assay kit (Millipore, Schwalbach, Germany).

3. Results

Protein kinase CK2 is implicated in the regulation of various signaling cascades in the cell. Very little is known about the regulation of CK2 by external signals. In the present study we asked whether insulin might have an influence on the CK2 activity in pancreatic β-cells. To address to this question Min6 cells were treated for 4 h with 100 nM insulin. Cells were extracted and CK2 activity was measured with the CK2-specific substrate peptide [14]. As shown in Fig. 1A, treatment of pancreatic β-cells with insulin led to a twofold increase in the CK2 kinase activity. Since this increase could be due to elevated levels of CK2 we analyzed CK2 subunits by Western blot. This Western blot of the cell extract revealed that equal amounts of CK2 subunits were present in the absence and in the presence of insulin. Thus, there seems to be a specific increase in the CK2 kinase activity.

To further analyze the contribution of CK2 in the response of pancreatic β-cells to the insulin pathway, we treated the β-cells with a specific inhibitor of CK2, namely quinalizarin [6]. Protein kinase activity was then analyzed in the presence or absence of quinalizarin. In order to exclude any cell line specific contribution

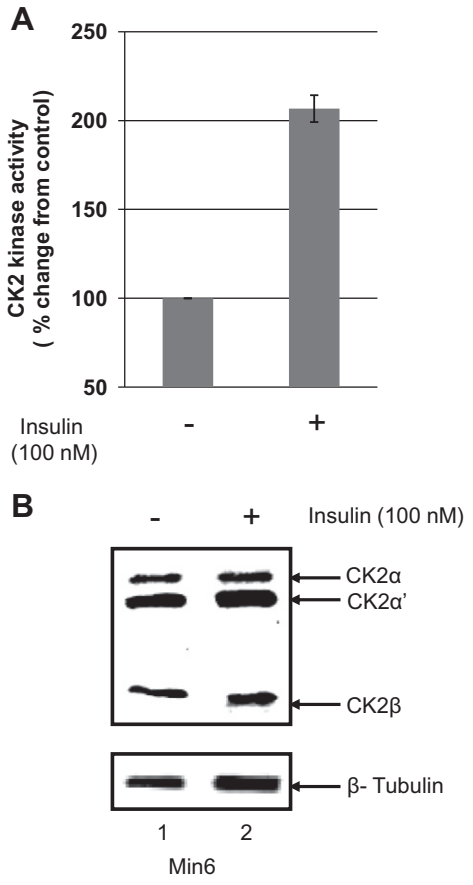

Fig. 1. Insulin treatment modulates the kinase activity of protein kinase CK2 without affecting its protein level. Min6 cells were incubated with HEPES balanced Krebs–Ringer Buffer (KRBH) supplemented with 0.1% BSA in the absence of glucose for 30 min, and in the presence of 2 mM glucose for a further 4 h, followed by a 20-min stimulation with (+) or without (−) 100 nM insulin. The cells were immediately harvested and subjected to a kinase assay and Western blot analysis. Kinase activity in non-insulin treated cells was set 100%. A histogram representative of three individual experiments is shown (A). Immunoblots with anti-CK2α, α′, β and anti-β-tubulin antibodies are shown (B).

to the CK2 inhibition we used two different pancreatic β-cell lines, βTC-3 or Min6 cells which were incubated with 50 μM quinalizarin for 24 h. Cells were lysed and the cell extract was analyzed for CK2 protein kinase activity using a highly specific CK2 peptide substrate. As shown in Fig. 2A, in both cell lines the kinase activity was reduced to about 50–60% in the presence of 50 μM quinalizarin. A Western blot analysis (Fig. 2B) further revealed no differences in the amount of CK2α and α′ in the presence or absence of quinalizarin.

Next, we asked whether this reduction in the kinase activity might have an influence on the insulin secretion of the pancreatic β-cells. We treated Min6 cells with 50 μM quinalizarin or left them untreated. Supernatants were collected and the insulin content was measured with a mouse insulin ELISA kit. As shown in Fig. 3, inhibition of the CK2 kinase activity resulted in an elevated level of insulin in the culture medium. Thus, these data show an autoregulatory loop in the regulation of insulin production in pancreatic β-cells and moreover, they suggest CK2 as a mediator of this autoregulatory loop.

4. Discussion

Multiple signals of different origin guarantee appropriate β-cell function under both basal and glucose-stimulated conditions. These signals include hormones, vitamins and nutrients. An autocrine effect of secreted insulin on β-cell function is still a matter of debate. With regard to the effect of insulin upon insulin secretion a negative feedback [3,24] or a positive feedback [23,31] or no effect at all [32] have been reported. Our present data support the idea about a negative feedback regulation of insulin on insulin production. It is clear from a number of recent papers that insulin

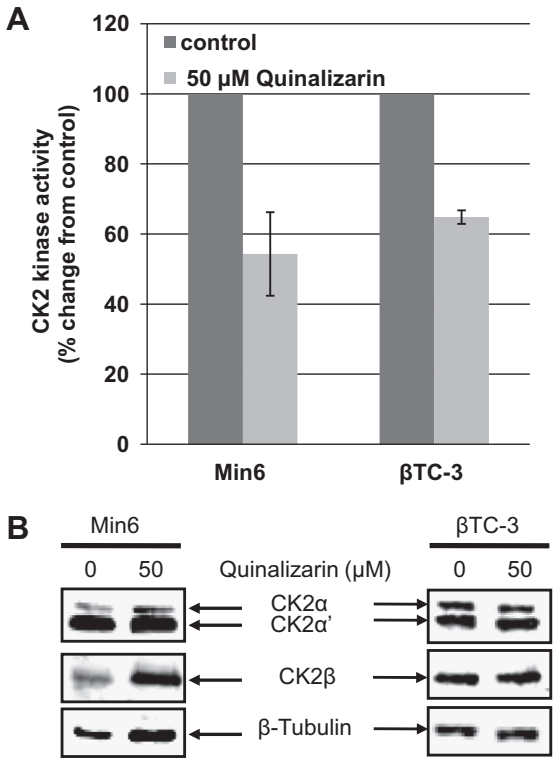

Fig. 2. Inhibition of protein kinase CK2 activity in pancreatic β-cells after treatment with quinalizarin. (A) CK2 activity was measured in extracts from Min6 and βTC-3 cells treated with 0 and 50 μM quinalizarin for 24 h by the incorporation of [^{32}P]-phosphate into a synthetic substrate peptide. (B) Western blot analysis using anti-CK2α, α′, β and anti-β-tubulin antibodies (loading control) showed the amount of CK2α, CK2α′ and CK2β in the quinalizarin treated Min6 and βTC-3 cells.

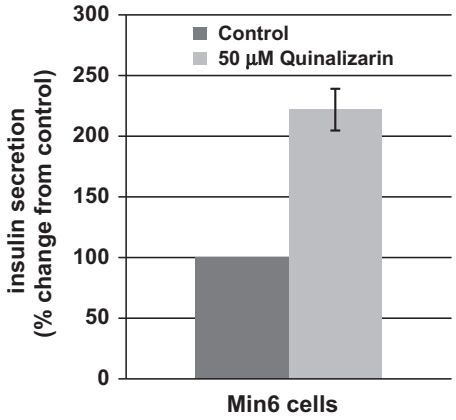

Fig. 3. Effect of quinalizarin on insulin secretion in Min6 cells. Min6 cells cultured in a 24-well plate were pre-incubated with normal growth medium with or without 50 μM quinalizarin overnight. After washing, cells were incubated in HEPES balanced Krebs–Ringer Buffer (KRBH) containing 0.1% BSA and supplemented with or without 50 μM quinalizarin for 4 h. The medium was replaced by fresh KRBH containing 0.1% BSA for another 20 min. The culture medium was finally collected, and the insulin content was measured by a rat/mouse insulin ELISA Kit (Millipore). The experiment was performed in triplicate, and the data are presented as percentage of insulin release in the absence of quinalizarin.

plays a role in the regulation of gene transcription, translation and Ca^{2+} signaling [10,25,30]. One possible source for the controversial results may be the question whether the observed insulin effect upon β-cell function is a direct one or rather secondary, mediated by other factors. Here we identified protein kinase CK2 as a new factor, which was implicated in insulin mediated signaling in β-cells. Insulin binds to the insulin receptor of β-cells. The complete physiological consequences of insulin receptor activation of β-cells have yet to be completely elucidated, but at least one effect is initiation of protein synthesis [17]. However, we showed here that the amount of the CK2 subunits was not altered after insulin stimulation. Instead the protein kinase activity increased after insulin treatment. One of the substrates that is phosphorylated by protein kinase CK2 is Pdx-1. Pdx-1 is a well studied transcription factor, which is critical to both β-cell development and function. Many studies indicated that the expression and/or activation of Pdx-1 in β-cells are reduced under diabetic conditions. On the other hand Pdx-1 together with other transcription factors regulates both,

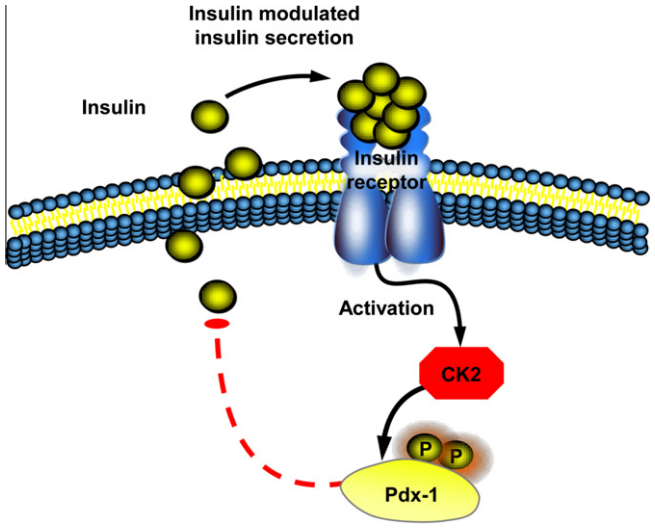

Fig. 4. Schematic representation of a putative negative feedback of insulin regulated insulin secretion via CK2 phosphorylation of the Pdx-1 protein. Black arrow indicates stimulation effect while red arrow means inhibitory effect.

insulin gene transcription and insulin secretion. Pdx-1 is a phosphoprotein and it was assumed that the transcription factor activity of Pdx-1 might be regulated by phosphorylation (for review see: [1]. Recently, we discovered that Pdx-1 is a substrate for protein kinase CK2 [21]. The CK2 phosphorylation sites were localized at threonine 231 and serine 232 on the polypeptide chain of Pdx-1. By mutating the serine and threonine residues into alanine residues, which can not be phosphorylated by CK2, it was shown that this mutant Pdx-1 led to an elevated transcription of the insulin gene. Here we showed that insulin stimulated the kinase activity of CK2. Together with our previous data this would suggest a down-regulation of the insulin production as illustrated in Fig. 4. Consequently, inhibition of the CK2 kinase activity resulted in an elevated level of insulin secretion as shown here.

Acknowledgment

Rui Meng is supported by a scholarship for postgraduates from the China Scholarship Council under the supervision of Dr. Gang Wu from the Cancer Center of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, 430023 Wuhan, Hubei, PR China. We thank Nathaniel Saidu for his help with the manuscript. This study was supported by the program “Das zuckerkranke Kind” from the “Deutsche Diabetes Gesellschaft” and by Homfor T 201 000 378 to Claudia Götz.

References

[1] F. Al-Quobaili, M. Montenarh, Pancreatic duodenal homeobox factor-1 and diabetes mellitus type 2, Int. J. Mol. Med. 21 (2008) 399–404.
[2] R. An, X.G. daSilva, H.X. Hao, F. Semplici, J. Rutter, G.A. Rutter, Regulation by Per-Arnt-Sim (PAS) kinase of pancreatic duodenal homeobox-1 nuclear import in pancreatic beta-cells, Biochem. Soc. Trans. 34 (2006) 791–793.
[3] C.A. Aspinwall, W.J. Qian, M.G. Roper, R.N. Kulkarni, C.R. Kahn, R.T. Kennedy, Roles of insulin receptor substrate-1, phosphatidylinositol 3-kinase, and release of intracellular Ca^{2+} stores in insulin-stimulated insulin secretion in beta-cells, J. Biol. Chem. 275 (2000) 22331–22338.
[4] M.J. Boucher, L. Selander, L. Carlsson, H. Edlund, Phosphorylation marks IPF1/PDX1 protein for degradation by glycogen synthase kinase 3-dependent mechanisms, J. Biol. Chem. 281 (2006) 6395–6403.
[5] M.J. Boucher, M. Simoneau, H. Edlund, The homeodomain-interacting protein kinase 2 regulates insulin promoter factor-1/pancreatic duodenal homeobox-1 transcriptional activity, Endocrinology 150 (2009) 87–97.
[6] G. Cozza, M. Mazzorana, E. Papinutto, J. Bain, M. Elliott, M.G. Di, A. Gianoncelli, M.A. Pagano, S. Sarno, M. Ruzzene, R. Battistutta, F. Meggio, S. Moro, G. Zagotto, L.A. Pinna, Quinalizarin as a potent, selective and cell-permeable inhibitor of protein kinase CK2, Biochem. J. 421 (2009) 387–395.
[7] R. D'Ambra, M. Surana, S. Efrat, R.G. Starr, N. Fleischer, Regulation of insulin secretion from beta-cell lines derived from transgenic mice insulinomas resembles that of normal beta-cells, Endocrinology 126 (1990) 2815–2822.
[8] M. Faust, N. Schuster, M. Montenarh, Specific binding of protein kinase CK2 catalytic subunits to tubulin, FEBS Lett. 462 (1999) 51–56.
[9] Y. Gao, J. Miyazaki, G.W. Hart, The transcription factor Pdx-1 is post-translationally modified by O-linked N-acetylglucosamine and this modification is correlated with its DNA binding activity and insulin secretion in min6 beta-cells, Arch. Biochem. Biophys. 415 (2003) 155–163.
[10] J.C. Henquin, Regulation of insulin secretion: a matter of phase control and amplitude modulation, Diabetologia 52 (2009) 739–751.
[11] H. Ishihara, T. Asano, K. Tsukuda, H. Katagiri, K. Inukai, M. Anai, M. Kikuchi, Y. Yazaki, J.I. Miyazaki, Y. Oka, Pancreatic beta cell line MIN6 exhibits characteristics of glucose metabolism and glucose-stimulated insulin secretion similar to those of normal islets, Diabetologia 36 (1993) 1139–1145.
[12] A. Kishi, T. Nakamura, Y. Nishio, H. Maegawa, A. Kashiwagi, Sumoylation of Pdx1 is associated with its nuclear localization and insulin gene activation, Am. J. Physiol. Endocrinol. Metab 284 (2003) E830–E840.
[13] J.K. Klarlund, M.P. Czech, Insulin-like growth factor I and insulin rapidly increase casein kinase II activity in BALB/c 3T3 fibroblasts, J. Biol. Chem. 263 (1988) 15872–15875.
[14] E.A. Kuenzel, E.G. Krebs, A synthetic peptide substrate specific for casein kinase II, Proc. Natl. Acad. Sci. USA 82 (1985) 737–741.
[15] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (1970) 680–682.
[16] P. Lebrun, M.R. Montminy, O.E. Van, Regulation of the pancreatic duodenal homeobox-1 protein by DNA-dependent protein kinase, J. Biol. Chem. 280 (2005) 38203–38210.
[17] I.B. Leibiger, B. Leibiger, T. Moede, P.O. Berggren, Exocytosis of insulin promotes insulin gene transcription via the insulin receptor/PI-3 kinase/p70 s6 kinase and CaM kinase pathways, Mol. Cell 1 (1998) 933–938.
[18] D.W. Litchfield, Protein kinase CK2: structure, regulation and role in cellular decisions of life and death, Biochem. J. 369 (2003) 1–15.
[19] M. Lu, C. Miller, J.F. Habener, Functional regions of the homeodomain protein IDX-1 required for transactivation of the rat somatostatin gene, Endocrinology 137 (1996) 2959–2967.
[20] F. Meggio, L.A. Pinna, One-thousand-and-one substrates of protein kinase CK2?, FASEB J 17 (2003) 349–368.
[21] R. Meng, F. Al-Quobaili, I. Müller, C. Götz, G. Thiel, M. Montenarh, CK2 phosphorylation of Pdx-1 regulates its transcription factor activity, Cell Mol. Life Sci. 67 (2010) 2481–2489.
[22] A.-M. Mes, J.A. Hassell, Polyoma vrial middle T-antigen is required for transformation, J. Virol. 42 (1982) 621–629.
[23] S.J. Persaud, H. Asare-Anane, P.M. Jones, Insulin receptor activation inhibits insulin secretion from human islets of Langerhans, FEBS Lett. 510 (2002) 225–228.
[24] M.G. Roper, W.J. Qian, B.B. Zhang, R.N. Kulkarni, C.R. Kahn, R.T. Kennedy, Effect of the insulin mimeticL-783,281 on intracellular Ca2+ and insulin secretion from pancreatic beta-cells, Diabetes 51 (Suppl. 1) (2002) S43–S49.
[25] S. Shao, Z. Fang, X. Yu, M. Zhang, Transcription factors involved in glucose-stimulated insulin secretion of pancreatic beta cells, Biochem. Biophys. Res. Commun. 384 (2009) 401–404.
[26] J. Sommercorn, J.A. Mulligan, F.J. Lozeman, E. Krebs, Activation of casein kinase II in response to insulin and to epidermal growth factor, Proc. Natl. Acad. Sci. USA 84 (1987) 8834–8838.
[27] N.A. St-Denis, D.W. Litchfield, From birth to death: the role of protein kinase CK2 in the regulation of cell proliferation and survival, Cell Mol. Life Sci. 66 (2009) 1817–1829.
[28] M. Stoffel, R. Stein, C.V. Wright, R. Espinosa III, M.M. Le Beau, G.I. Bell, Localization of human homeodomain transcription factor insulin promoter factor 1 (IPF1) to chromosome band 13q12.1, Genomics 28 (1995) 125–126.
[29] J.H. Trembley, G. Wang, G. Unger, J. Slaton, K. Ahmed, CK2: a key player in cancer biology, Cell Mol. Life Sci. 66 (2009) 1858–1867.
[30] G. Xu, G. Kwon, C.A. Marshall, T.A. Lin, J.C. Lawrence Jr., M.L. McDaniel, Branched-chain amino acids are essential in the regulation of PHAS-I and p70 S6 kinase by pancreatic beta-cells. A possible role in protein translation and mitogenic signaling, J. Biol. Chem. 273 (1998) 28178–28184.
[31] W.S. Zawalich, G.J. Tesz, K.C. Zawalich, Are 5-hydroxytryptamine-preloaded beta-cells an appropriate physiologic model system for establishing that insulin stimulates insulin secretion?, J Biol. Chem. 276 (2001) 37120–37123.
[32] W.S. Zawalich, K.C. Zawalich, Effects of glucose, exogenous insulin, and carbachol on C-peptide and insulin secretion from isolated perifused rat islets, J. Biol. Chem. 277 (2002) 26233–26237.