Interleukin-1β activates a short STAT-3 isoform in clonal insulin-secreting cells

Nicholas M. Morton[a], Rolf P. de Groot[b], Michael A. Cawthorne[a], Valur Emilsson[a,*]

[a] *Clore Laboratory, The University of Buckingham, Hunter Street, Buckingham MK18 1EG, UK*
[b] *Department of Pulmonary Diseases, University Hospital Utrecht, P.O. Box 85500, 3508 GA Utrecht, The Netherlands*

Received 13 November 1998

Abstract Interleukin-1β (IL-1β) is a potent inflammatory cytokine involved in type 1 diabetes and acts through defined IL-1β signaling pathways. In the present work we describe induction of DNA binding activity to signal transducer and activator of transcription (STAT) in response to IL-1β in clonal insulin-secreting cells. Moreover, IL-1β activates a short isoform of STAT-3 that potently stimulates transcription. Immunoprecipitation studies reveal an interaction between the activated STAT-3 and the IL-1 receptor accessory protein indicating an association between the two signaling pathways. This may be a novel point of transduction cross talk and an additional mechanism utilised by IL-1β in the pancreatic β-cell during the process of type 1 diabetes.

Key words: Interleukin 1β; Signal transducer and activator of transcription 3; Clonal β-cell; Interleukin 1 receptor accessory protein

1. Introduction

Interleukin-1β (IL-1β) is a potent pro-inflammatory cytokine that signals through the type I interleukin 1 receptor (IL-1RI) [1,2]. IL-1RI and its essential co-receptor accessory protein (IL-1RAcP) [3] belong to the immunoglobulin superfamily of receptors and are distinct from other cytokine receptors [2]. IL-1β is recognised as one of the instrumental factors in pancreatic islet β-cell destruction during autoimmune pancreatitis and type 1 diabetes [4]. IL-1β mediates the intracellular activation through the IL-1RI-associated kinases, death domain proteins, the sphingomyelin/ceramide cascade, the nuclear factor NF-κB, Jun N-terminal kinase and mitogen-activated protein kinase signaling pathways [1,5–8]. Beta-cell damage occurs after increased production of nitric oxide and inflammatory mediators due to elevated expression of inducible nitric oxide synthase [9] and cyclooxygenase 2 [10]. This leads to abrogation of glycolytic flux [11], mitochondrial respiratory processes [12] and apoptotic cell death [13,14].

The Janus kinase (JAK) and signal transducer and activators of transcription (STAT) signaling pathway is associated with cytokines and growth factors that signal through the type I cytokine receptor superfamily [15–17]. To date, there is little evidence suggesting cross talk between the IL-1β and JAK/STAT signaling pathways. There are no obvious JAK or STAT binding motifs within the IL-1RI or the IL-1RAcP, and this could indicate that adapter proteins are necessary to couple these two pathways. In the present study we provide evidence for the activation of a short isoform of STAT-3 in response to IL-1β in the pancreatic β-cell model RINm5F. Furthermore, we find an interaction of the IL-1RAcP with STAT-3 that supports a rapid recruitment and activation of the JAK/STAT pathway after binding of IL-1β to the IL-1RI/IL-1RAcP complex. Since the susceptibility of the pancreatic β-cell to IL-1β-mediated damage is influenced by its metabolic status [18,19], we also determined the effect of mimicking increased metabolic activity on this signaling process. We propose that this novel point of cross talk between two distinct cytokine signaling pathways may be an additional mechanism used by IL-1β during the process of pancreatic β-cell destruction in type 1 diabetes.

2. Materials and methods

2.1. Cell culture and transient transfection

RINm5F cells were routinely cultured as described previously [20]. Cells were exposed to recombinant human IL-1β (Calbiochem, Nottingham, UK), glucagon-like peptide-1 (GLP-1) (Bachem, Saffron Walden, UK), or forskolin and isobutyl methyl xanthine (IBMX) (Alexis Corp., Nottingham, UK) for the indicated times and concentrations in serum-free RPMI 1640 medium. For transfection experiments, interferon-γ-activated sequence (GAS)-chloramphenicol acetyltransferase (CAT) containing four copies of the human FcγRI GAS (5′-AGC TTG AGA TGT ATT TCC CAG AAA AGA-3′), interferon-γ/interleukin-6 response element (IRE)-CAT containing four copies of the human intercellular adhesion molecule 1 (ICAM-1)-CAT (5′-AGC TTA GTT TCC GGG AAA GCA C-3′), and β-casein-CAT containing four copies of the β-casein STAT binding site (5′-AGC TTA GAT TTC TAG GAA TTC AAA TCA-3′) were used. STAT binding sites were cloned into the TK-CAT vector pBLCAT2 [21]. Cells were split 1:3 and 24 h later transfected with 10 μg supercoiled plasmid DNA by the DEAE-dextran technique. Cells were serum starved for 16 h, after which the cells were either stimulated with IL-1β (50 pg/ml) or left untreated for 12 h and subsequently harvested for CAT assay as described previously [22].

2.2. Mobility shift assay and supershift

Gel shift assay was performed with short STAT consensus binding element (underlined) from the interferon response factor 1 (IRF-1) promoter, top strand 5′-GCC TGA TTT CCC CGA AAT GAC GGC-3′, c-fos promoter, high affinity SIS inducible element (m67SIE), top strand 5′-CAT TTC CCG TAA ATC AT-3′ and rat β-casein promoters top strand 5′-GGA CTT CTT GGA ATT AAG GGA-3′, end-labelled with [γ-^{32}P]ATP (Amersham, Little Chalfont, UK). Supershift experiments were performed with anti-STAT-1 (C-136X), -3 (C-20X), -5a (L-20X), -5b (C-17X) (Santa Cruz, CA, USA) and anti-STAT-1 N-terminal (G16920), anti-STAT-3 N-terminal (S21320), STAT-2 (S21220), and STAT-6 (S25420) (Transduction

*Corresponding author. Fax: (44) (1280) 822245.
E-mail: valur.emilsson@buckingham.ac.uk

Abbreviations: IL-1β, interleukin-1β; IL-1RI, interleukin-1 receptor type I; IL-1RAcP, interleukin-1 receptor accessory protein; STAT, signal transducer and activator of transcription; JAK, Janus kinase; IBMX, isobutyl methyl xanthine; GAS, interferon-γ-activated sequence; CAT, chloramphenicol acetyltransferase; IRE, interferon-γ/interleukin-6 response element; ICAM, intercellular adhesion molecule; IRF-1, interferon response factor-1; SIE, SIS inducible element; cAMP, cyclic adenosine monophosphate; GLP-1, glucagon-like peptide 1

PII: S0014-5793(98)01623-8

Labs, Lexington, KY, USA) by pre-incubation of nuclear extracts for 15 min at room temperature, or 30 min on ice before addition of probe. Nuclear extracts were prepared as described previously [23].

2.3. Immunoprecipitation and Western blots

The cells were lysed and the lysate rocked for 30 min at 4°C. For phospho (Y705) STAT-3, the lysate was cleared by adding 30 μl protein G-Sepharose (Pharmacia) for 1 h, spun down and the supernatant incubated overnight at 4°C with 1 μg/condition of a rabbit polyclonal anti-phospho (Y705) STAT-3 antibody (New England Biolabs). Goat anti-rabbit IgG (Transduction Labs) was then incubated for 30 min and the complexes pulled down with protein G-Sepharose beads after a 30 min rock. Immunoprecipitates were then washed and loaded on 10% SDS-PAGE system. The blots were probed with either a mouse anti-IL-1RAcP antibody (Transduction Labs) or STAT-3 N-terminal-specific antibodies (Transduction Labs) and detected with an anti-mouse horseradish peroxidase-conjugated secondary antibody according to the manufacturer's instructions with an ECL kit (Amersham). Western blot analysis was performed as described previously [23].

3. Results and discussion

IL-1β (50 pg/ml) induced DNA binding activity to the GAS element from the IRF-1 promoter (Fig. 1) which was detectable by 5 min. Binding of the STAT factor reached a maximum at 15–20 min and returned to basal within 30 min consistent with the kinetics of rapid STAT factor activation. IL-1β induced STAT DNA binding activity in nuclear extracts of the clonal insulin-secreting cell line RINm5F at concentrations that are within the exposure range that promotes islet β-cell damage [9–14,18,19]. Thus, the induction of this single DNA binding complex was observed over the concentration range of 0.5–500 pg/ml IL-1β with saturation of binding at 5 pg/ml during a 15 min incubation with the IRF-1 probe (Fig. 1). Using the IRF-1 probe, we were unable to supershift the complex with C-terminal antibodies to STAT-1, -2, -3, -5a, -5b or -6 (Fig. 1, lower panel). We also found that IL-1β (50 pg/ml) induces binding to the DNA m67SIE STAT element from the c-fos promoter (Fig. 2), a probe known to bind STAT-3α and STAT-3β avidly. The single complex binding to the m67SIE probe was readily supershifted with an anti-N-terminal STAT-3 antibody (Fig. 2). The STAT m67SIE complex was not supershifted with C-terminal STAT-3 antibody nor was there any induction of DNA binding activity to a β-casein oligonucleotide probe in RINm5F nuclear extracts implying that STAT-5 is not a target for IL-1β (data not shown).

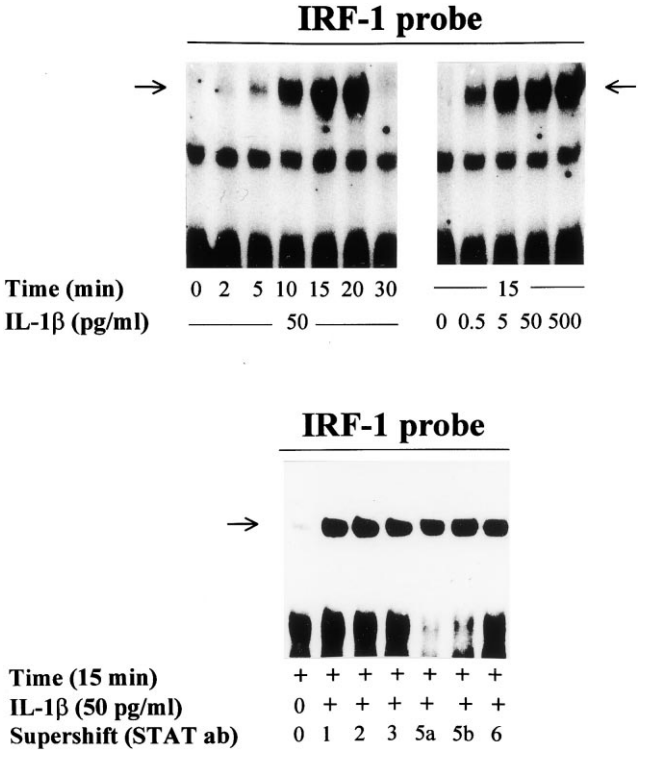

Fig. 1. IL-1β induces time- and dose-dependent DNA binding activity on the IRF-1 STAT consensus. IL-1β (50 pg/ml)-induced STAT DNA binding to the IRF-1 is observed by 5 min, peaks at 15–20 min and returns to basal by 30 min (upper panel). The STAT DNA binding is observed over the concentration range of 0.5–500 pg/ml (upper panel). Supershift analyses of IL-1β (50 pg/ml)-induced STAT DNA binding to the IRF-1 with C-terminal antibodies to STAT-1, -2, -3, -5a, -5b and -6 are shown in the bottom panel. The arrows point at the STAT DNA complex.

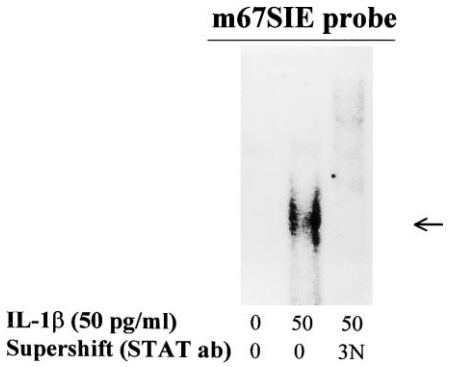

Fig. 2. IL-1β induces STAT-3 DNA binding activity. An appropriate time (15 min) and dose of IL-1β (50 pg/ml) was selected from results on the IRF-1 probe, to study the effects of IL-1β on m67SIE binding activity. IL-1β (50 pg/ml) induced the STAT DNA binding activity of the m67SIE probe that is known to bind STAT-3 avidly. The arrow points at the STAT DNA band shift.

Nuclear extracts from 50 pg/ml IL-1β-treated RINm5F cells were also subjected to Western blot and probed with an antibody to the tyrosine phosphorylated form of STAT-3 (anti-phospho (Y705) STAT-3) and an N-terminal-directed STAT-3 antibody. This revealed that IL-1β induces tyrosine phosphor-

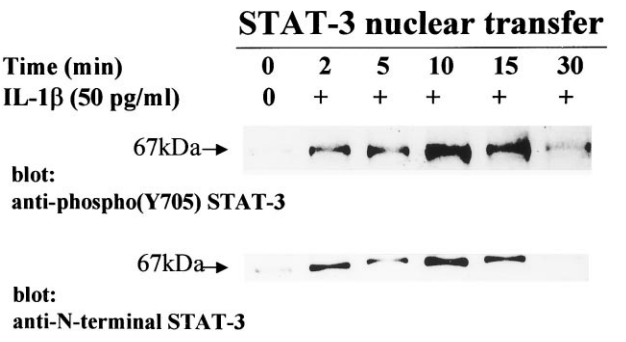

Fig. 3. IL-1β causes a time-dependent induction of a STAT-3 immunoreactive protein in RINm5F nuclear extracts. RINm5F were preincubated in serum-free medium for 24 h and then exposed to IL-1β (50 pg/ml) for the times indicated below the lanes. Time-dependent induction of a protein complex (~67 kDa) that cross-reacts with a phospho (Y705) STAT-3 antibody. Time-dependent induction of a STAT-3 N-terminal immunoreactive protein that co-migrates with the tyrosine phosphorylated STAT-3 species at approximately 67 kDa.

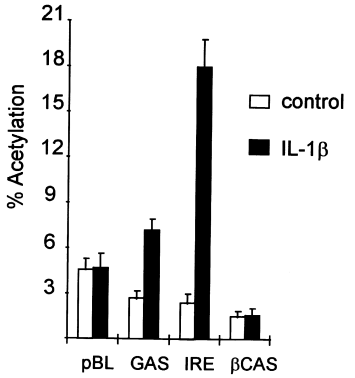

Fig. 4. IL-1β induces transcriptional activation from the IRE-CAT. RINm5F cells were transiently transfected with reporter plasmids driven by the STAT consensus promoter element indicated below the axis. After a period of serum starvation, the RINm5F were exposed to IL-1β (50 pg/ml) for 12 h and then assessed for induction of CAT activity. The IRE-CAT reporter construct was strongly induced whereas a slight induction at the GAS-CAT and no induction from a control plasmid pBL or βCAS-CAT constructs were observed.

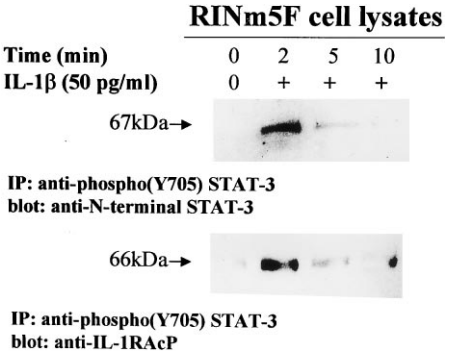

Fig. 5. IL-1β induces the interaction of activated STAT-3 with the IL-1RAcP. RINm5F cells were treated with IL-1β (50 pg/ml) for the indicated time and then lysed. Immunoprecipitation was performed on whole cell lysates with an anti-phospho STAT-3 Y705 antibody. Identical immunoprecipitates were then probed with either an N-terminal directed STAT-3 antibody or an anti-IL-1RAcP monoclonal antibody on separate gels. IL-1β treatment increases the association with a peak at 2 min that rapidly declines by 10 min.

ylation of a single STAT-3 isoform (Fig. 3). This band co-migrated with and followed an identical induction pattern to a STAT-3 N-terminal immunoreactive species with an apparent molecular weight of 67 kDa (Fig. 3). The fact that a C-terminal directed STAT-3 antibody does not supershift the IL-1β-induced DNA binding activity (anti-STAT-3 epitope 750–769) (Fig. 1) is consistent with the induction of a short STAT-3 isoform. Tyrosine phosphorylation and nuclear translocation (Fig. 3) correlated well with the time course for STAT DNA binding (Fig. 1).

In order to determine whether the observed activation of STAT DNA binding activity also results in transcriptional activation of STAT target genes, we performed transient transfection experiments in RINm5F cells (Fig. 4). IL-1β (50 pg/ml) caused a modest activation of a CAT reporter construct containing four copies of the human FcγRI GAS (GAS-CAT), which can bind STAT-1, -3 and -5. However, the activity of a CAT reporter construct containing four copies of the human ICAM-1 IRE (IRE-CAT), which can bind STAT-1 but has a greater affinity for STAT-3 [24], was strongly enhanced by IL-1β treatment. By contrast, no increase in CAT activity could be found in cells transfected with β-casein-CAT containing four copies of the β-casein STAT binding site, which can bind STAT-1 and efficiently binds STAT-5, or with the empty pBLCAT2 vector. Taken together, these results support the activation of a transcriptionally active STAT-3 or a STAT-3-related molecule after IL-1β treatment of RINm5F cells.

Since the induction of STAT-3 activation occurs rapidly, we reasoned that there might be an interaction with the ligand-receptor complex at a membrane proximal level. In order to address a possible point of interaction between the IL-1RI system and the activation of STAT-3, we performed immunoprecipitation studies with an antibody to the activated form of STAT-3 and probed with an antibody to the IL-1RAcP. This co-receptor is known to be essential for the full functioning capability of the IL-1RI complex [2]. We found that there was a rapid association of STAT-3 with IL-1RAcP that was maximal at 2 min and declined over the next 10 min (Fig. 5). STAT-3 recruitment to ligand-activated cytokine receptors is usually mediated through STAT SH2 domain binding to tyrosine phosphorylated motifs on the receptor [17] and direct interaction of STATs with JAK are also known to occur [25]. Our data indicate that a further level of association between activated STAT-3 and the IL-1RAcP may exist. The association could be a consequence of the interaction of other regions of the STAT-3 with the IL-1RAcP complex.

High IL-1β concentrations, prolonged duration of exposure and high glucose concentrations exacerbate the deleterious effects of IL-1β on the β-cell [18,19]. We tested whether the STAT activation mediated by IL-1β, presented in the current study, could be modulated by conditions that mimic increased metabolic activity using agents that increase intracellular cyclic adenosine monophosphate (cAMP) concentrations as occurs in normal pancreatic β-cells upon exposure to hormones that potentiate insulin secretion. GLP-1 (10 nM), an endogenously secreted peptide hormone that increases intracellular cAMP concentrations and stimulates insulin secretion in RINm5F [26] cells, caused a reduction in IL-1β (50 pg/ml)-mediated STAT-3 DNA binding (Fig. 6). Increasing the intracellular cAMP concentration with the adenylyl cyclase activator, forskolin (10 μM) and cAMP phosphodiesterase inhibitor

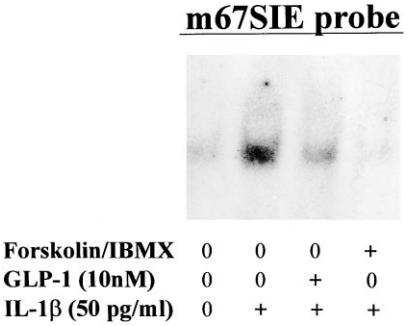

Fig. 6. Increasing intracellular cAMP attenuates the IL-1β-inducible STAT DNA binding. The cells were pre-incubated in serum-free medium and then given a second pre-incubation for 15 min with 10 nM GLP-1 or 10 μM forskolin/50 μM IBMX. IL-1β (50 pg/ml) was then added in the presence of the compounds and the nuclear extracts isolated. The nuclear extracts were then incubated with [γ-^{32}P]ATP end-labelled m67SIE probe and the complexes separated on 4% PAGE gels and analysed by autoradiography.

IBMX (50 μM) had a more pronounced effect. This may implicate a role for cAMP-dependent protein kinases in modulating IL-1β-mediated signaling. We do not know whether the STAT-3 mechanism represents the initiation of an early protective cellular mechanism or contributes to the cellular toxicity associated with inappropriate exposure to IL-1β. Our data indicate that acute elevation of β-cell metabolic activity could counteract IL-1β-mediated STAT signaling.

The events after IL-1β binding to its receptor have only recently begun to be elucidated [1–8]. Activation of the JAK/STAT pathway is associated with growing numbers of ligands [15–17]. To date, however, only one study has described an interaction of the IL-1β signaling pathway and a STAT-1-like mechanism [27]. We probed nuclear extracts of IL-1β-treated RINm5F with antibodies to STAT-1 N-terminus and phospho (Y701) STAT-1 and found that this complex is not induced in this cell line under the conditions used (data not shown). The STAT-3 isoform activation profile is somewhat different from the characterised short STAT-3β isoform. STAT-3β is a splice variant [22], which is functionally distinct from the full-length STAT-3α. Thus, STAT-3β has a higher DNA binding affinity but lacks the C-terminal transactivation domain that is believed to convey transcriptional activity to STAT-3α [28]. Indeed, STAT-3β can mediate dominant negative repression of transcription [22] or transcriptional activation [29] depending on the system studied. Thus, Sasse et al. [29] have shown that carboxy-terminal truncated STAT-3 proteins are unable to activate promoters in COS-7 cells, whilst in hepatoma cells (HepG2) they potently activate promoters and to the same extent as a full-length STAT-3. This would suggest that different mechanisms exist in different cell types to promote transactivation of STAT-3 that is not always dependent on the presence of the carboxy-terminal transactivation domain. The transactivation observed in response to IL-1β in the RINm5F cells implies that the short STAT-3 isoform described here has transcriptional activity in insulin-producing cells. Regarding whether the STAT-3 mechanism mediates a protective or destructive effect, it is of note that IRF-1 is known to have a crucial role in the initiation of apoptosis and cellular growth retardation [30]. Furthermore, co-operative interaction between STAT-3β and c-Jun [31], another IL-1β-responsive transcription factor that has been linked to apoptosis [32], has also been established. However, since STAT-3 activation has been associated with diverse and often opposing phenomena, such as transformation [33] or apoptosis and differentiation [34] depending on the system under study, the role of this signaling mechanism in the fate of the β-cell during type 1 diabetes will require further studies.

References

[1] Martin, M.U. and Falk, W. (1997) Eur. Cytokine Netw. 8, 5–17.
[2] Dinarello, C.A. (1994) FASEB J. 8, 1314–1325.
[3] Wesche, H., Kracht, M., Falk, W., Resch, K. and Martin, M.U. (1997) J. Biol. Chem. 272, 7727–7731.
[4] Mandrup-Poulsen, T., Bendtzen, K., Nerup, J., Dinarello, C.A., Svenson, M. and Nielsen, J.H. (1986) Diabetologia 29, 63–67.
[5] Muzio, M., Ni, J., Feng, P. and Dixit, V.M. (1997) Science 278, 1612–1615.
[6] Cao, Z.D., Xiong, J., Takeuchi, T., Kurama, T. and Goedel, D.V. (1996) Nature 383, 443–446.
[7] Welsh, N. (1996) J. Biol. Chem. 271, 8307–8312.
[8] Bagrodia, S., Derijard, B., Davis, R.J. and Cerione, R.A. (1995) J. Biol. Chem. 270, 27995–27998.
[9] Corbett, J.A., Wang, J.L., Sweetland, M.A., Lancaster, J.R. and McDaniel, M.L. (1992) J. Clin. Invest. 90, 2384–2391.
[10] Corbett, J.A., Kwon, G., Turk, J. and McDaniel, M.L. (1993) Biochemistry 32, 13767–13770.
[11] Ma, Z., Landt, M., Bohrer, A., Ramanadham, S., Kipnis, D.M. and Turk, J. (1997) J. Biol. Chem. 272, 17827–17835.
[12] Welsh, N., Eizirik, D.L., Bendtzen, K. and Sandler, S. (1991) Endocrinology 129, 3167–3173.
[13] Ankarcrona, M., Dypbukt, J.M., Brune, B. and Nicotera, P. (1994) Exp. Cell Res. 213, 172–177.
[14] Kaneto, H., Fujii, J., Seo, H.G., Matsuoka, T., Nakamura, M., Tatsumi, H., Yamasaka, Y., Kamada, T. and Taniguchi, N. (1995) Diabetes 44, 733–738.
[15] Ihle, J.N. (1996) Cell 84, 331–334.
[16] Darnell Jr., J.E. (1997) Science 277, 1630–1635.
[17] Pellegrini, S. and Dusanter-Fourt, I. (1997) Eur. J. Biochem. 248, 615–633.
[18] Palmer, J.P., Helqvist, S., Spinas, G.A., Molvig, J., Mandrup-Poulsen, T., Andersen, H.U. and Nerup, J. (1989) Diabetes 38, 1211–1216.
[19] Dunger, A., Schroder, D., Augstein, P., Witstruck, T., Wachlin, G., Vogt, B., Ziegler, S. and Schmidt, S. (1995) Acta Diabetol. 32, 217–224.
[20] Islam, M.S., Morton, N.M., Hanssen, A. and Emilsson, V. (1997) Biochem. Biophys. Res. Commun. 238, 851–855.
[21] Luckow, B. and Schutz, G. (1987) Nucleic Acids Res. 15, 5490.
[22] Caldenhoven, E., van Dijk, T.B., Solari, R., Armstrong, J., Raaijmakers, J.A.M., Lammers, J.W.J., Koenderman, L. and de Groot, R.P. (1996) J. Biol. Chem. 271, 13221–13227.
[23] Morton, N.M., Emilsson, V., Liu, Y.-L. and Cawthorne, M.A. (1998) J. Biol. Chem. 273, 26194–26201.
[24] Caldenhoven, E., van Dijk, T., Raaijmakers, J.A.M., Lammers, J.-W.J., Koenderman, L. and de Groot, R.P. (1995) J. Biol. Chem. 270, 25778–25784.
[25] Fujitani, Y., Hibi, M., Fukuda, T., Takahashi-Tezuka, M., Yoshida, H., Yamaguchi, T., Sugiyama, K., Yamanaka, Y., Nakajima, K. and Hirano, T. (1997) Oncogene 14, 751–761.
[26] Goke, R. and Conlon, J.M. (1988) J. Endocrinol. 116, 357–362.
[27] Tsukada, J., Waterman, W.R., Koyama, Y., Webb, A.C. and Auberon, P.E. (1996) Mol. Cell. Biol. 16, 2183–2194.
[28] Schaeffer, T.S., Sanders, L.K., Park, O.K. and Nathans, D. (1997) Mol. Cell. Biol. 17, 5307–5316.
[29] Sasse, J., Hemmann, U., Schwartz, C., Schniertshauer, U., Heesel, B., Landgraf, C., Schneidermergener, J., Heinrich, P.C. and Horn, F. (1997) Mol. Cell. Biol. 17, 4677–4686.
[30] Nguyen, H., Lin, R.T. and Hiscott, J. (1997) Oncogene 15, 1425–1435.
[31] Schaeffer, T.S., Sanders, L.K. and Nathans, D. (1995) Proc. Natl. Acad. Sci. USA 92, 9097–9101.
[32] Bossy-Wetzel, E., Bakiri, L. and Yaniv, M. (1997) EMBO J. 16, 1695–1709.
[33] Turkson, J., Bowman, T., Garcia, R., Caldenhoven, E., De-Groot, R.P. and Jove, R. (1998) Mol. Cell. Biol. 18, 2545–2552.
[34] Naka, T., Narazaki, M., Hirata, M., Matsumoto, T., Minamoto, S., Aono, A., Nishimoto, N., Kajita, T., Taga, T., Yoshizaki, K., Akira, S. and Kishimoto, T. (1997) Nature 387, 924–929.