In models of type 1 diabetes, cytokines induce pancreatic β-cell death by apoptosis. This process seems to be facilitated by a reduction in the amount of the islet-brain 1/JNK interacting protein 1 (IB1/JIP1), a JNK scaffold with an anti-apoptotic effect. A point mutation S59N at the N terminus of the scaffold, which segregates in diabetic patients, has the functional consequence of sensitizing cells to apoptotic stimuli. Neither the mechanisms leading to IB1/JIP1 down-regulation by cytokines nor the mechanisms leading to the decreased capacity of the S59N mutation to protect cells from apoptosis are understood. Here, we show that IB1/JIP1 stability is modulated by intracellular calcium. The effect of calcium depends upon JNK activation, which primes the scaffold for ubiquitination-mediated degradation via the proteasome machinery. Furthermore, we observe that the S59N mutation decreases IB1/JIP1 stability by sensitizing IB1/JIP1 to calcium- and proteasome-dependent degradation. These data indicate that calcium influx initiated by cytokines mediates ubiquitination and degradation of IB1/JIP1 and may, therefore, provide a link between calcium influx and JNK-mediated apoptosis in pancreatic β-cells.

Type 1 diabetes mellitus is characterized by the selective destruction of pancreatic β-cells with preservation of the α-cells (glucagon-secreting), δ-cells (somatostatin-secreting) and PP-cells (pancreatic polypeptide secreting) (1). Accumulating evidence has implicated cytokines as key mediators of β-cell killing in rodent models of type 1 diabetes mellitus (2–5) and in human islet preparation (6) by apoptosis. The intra-islet release of IL-1β, tumor necrosis factor-α, and IFN-γ by activated mononuclear cells recruits into β-cells a highly complex network of signaling and effector molecules that have a decisive impact on cell fate. Among the demonstrated signaling molecules that transduce cytokine signaling in cultured β-cells, the transcription factor NF-κB and the mitogen activated protein kinase c-jun N-terminal kinase (JNK) play a major role in the induction of apoptosis (7–11). Recently it was shown that glucose-induced human β-cell apoptosis is blocked by IL-1 receptor antagonist and that the source of IL-1β is the β-cell itself, indicating that immunological and metabolic stimuli converge on common effector pathways leading to β-cell failure in both main types of diabetes (12).

In β-cells, JNK is responsive to cytokines probably through a transduction complex including the upstream mitogen activated protein kinase kinase kinase and mitogen activated protein kinase kinase kinase elements (13). The scaffold protein IB1/JIP1 ensures the formation, compartmentalization, and specificity of this physically ordered signaling module, so that a defined pool of JNK might be recruited by specific physiological stimuli (such as cytokines) but protected from activation by irrelevant ones (14). At the same time, IB1/JIP1 exerts an anti-apoptotic function probably by controlling the access of the activated JNKs to their downstream targets (13). This might be achieved by retaining JNK in specific subcellular localizations (mainly cytoplasmic) and preventing its access to some of its substrates, e.g. the nuclear transcription factor c-Jun or activating transcription factor-2. Indeed, the binding of IB1/JIP1 to JNK through the JNK-binding domain, a domain shared by many other substrates such as IRS-1 or c-Jun (15), is 100 times stronger than the interaction of JNK to its other substrates (16). This strong affinity of IB1/JIP1 for JNK effectively anchors JNK in the cytoplasm and prevents its interaction through the JNK-binding domain with most of its downstream substrates (including cytoplasmic ones) and their subsequent activation. In this model, degradation of IB1/JIP1 would free JNK and allow it both to enter the nucleus and to access its substrates, including c-Jun, activating transcription factor-2, and others. This event would seem to be a prerequisite for JNK-induced apoptosis.

The rapid JNK activation induced by treatment with cytokines is associated with a delayed reduction in the IB1/JIP1 content of β-cells, followed by apoptosis (13). The sensitivity of β-cells to apoptosis is even increased if IB1/JIP1 is mutated (S59N). This mutation was previously linked to a familial type 2 diabetes (17). These data indicate that the regulation of the IB1/JIP1 level might be important in deciding the cell-death or survival response.

The mechanism leading to a reduction of IB1/JIP1 content in response to cytokines is unclear, but could be linked to calcium-entry, as cytokines (IL-1β + IFN-γ) were also shown to mediate a...
low voltage-activated Ca<sup>2+</sup>- current (18). The objective of this study was to characterize and identify the protease(s) responsible for the cytokine-induced down-regulation of IB1/JIP1 in β-cells. Two major proteolytic pathways are known to operate in mammalian cells, the proteasome and the cysteine proteases, including the caspases, the calpains, and the lysosomal acidic cathepsins. Among them, calpains and some caspases are calcium-dependent. We show that calcium influx initiates ubiquitination and degradation of IB1/JIP1, and that this process seems to control the sensitivity of pancreatic β-cells to apoptotic stimuli. We also observed that the S59N point mutation in IB1/JIP1, linked to type 2 diabetes, sensitizes IB1/JIP1 to the calcium- and proteasome-dependent degradation.

**EXPERIMENTAL PROCEDURES**

**Plasmids, Peptides, and Antibodies**—The rat IB1 sequence was cloned into the expression vector pBK (Stratagene, La Jolla, CA), the mutated form (S59N), and the N-terminal isoform (M101) of the protein. Anti-IB1 antibody raised against amino acid 1–280 of the protein has been described (8). Anti-FLAG agarose resin and anti-tubulin antibodies were purchased from Sigma. The JNK1, mutated JNK1, and SH3 peptides were designed by us and synthesized by Auspep (Parkville, Australia).

**Cell Lines**—The HeLa cell line and the insulin-secreting βTC-3 cell line were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% nonfat dried milk powder, 10 mM sodium pyruvate, and 2 mM/liter glutamine. The calcium chelator BAPTA/AM was added at a concentration of 10 μM 60 min before the addition of 1 μM calcium ionophore A23187 (Calbiochem, San Diego, CA). The calpain inhibitor calpastatin was added at a concentration of 1 μM 60 min before the addition of the cytokines (10 ng/ml of IL-1β, 10 ng/ml of tumor necrosis factor-α, and 100 units/ml of IFN-γ) and the proteasome-inhibitor lactacystin was added at a concentration of 20 μM 60 min before the addition of the calcium ionophore A23187. The calpain inhibitor calpastatin was added at a concentration of 1 μM 60 min before the addition of the cytokines (10 ng/ml of IL-1β, 10 ng/ml of tumor necrosis factor-α, and 100 units/ml of IFN-γ) and the proteasome-inhibitor lactacystin was added at a concentration of 20 μM 60 min before the addition of the calcium ionophore A23187.

**RESULTS**

**Calcium Ionophore-induced IB1/JIP1 Degradation and βTC-3 Cell Apoptosis**—Cytokines induce pancreatic β-cell apoptotic signaling. Chronic exposure to cytokines reduces the level of IB1/JIP1 in the βTC-3 cell line and increases the apoptotic rate (13). Exposure to cytokines was shown recently to induce an increase in the basal cytoplasmic free calcium concentration through the low voltage-activated Ca<sup>2+</sup>- channels, which was associated with apoptosis (18). To determine whether increased intracellular calcium concentration and IB1/JIP1 degradation were related, we exposed the βTC-3 cell line to 1 μM calcium ionophore A23187 for 20 h, after which the IB1/JIP1 content was determined by Western blotting (Fig. 1A). The upper band is the full-length protein and the lower one is most likely the product of a translation beginning at Met-101 (20). Compared with control βTC-3 cells, A23187 led to a 10-fold decrease in IB1/JIP1 content (both full-length and Met-101 forms), which is equivalent to the down-regulation induced by a 48-h exposure to cytokines (Fig. 1A).

We also evaluated the number of apoptotic β-cells with a combination of propidium iodide and Hoechst 33342 nuclear staining (19). Culture in the presence of A23187 (24 h) or cytokines (48 h) induced in βTC-3 cells a 10-fold and an 8-fold increase in apoptotic rate, respectively (Fig. 1B). When exposure to ionophore was extended to 48 h, a massive cell death was observed (>50%), which led us to perform the following experiments over a period of 20 h.

**BAPTA-AM** was used to chelate intracellular calcium to confirm that the ionophore-induced degradation of IB1/JIP1 was calcium-dependent. BAPTA-AM is a selective cell-permeable calcium chelator which is a structural analogue of EGTA. When BAPTA-AM enters cells, the four acetoxyethyl ester (-AM) groups are cleaved off by endogenous intracellular esterases, making the BAPTA molecule negatively charged and trapping it intracellularly in the cytosol. Because BAPTA was toxic for βTC-3 cells and induced a rapid and massive cell death, we transfected HeLa cells with an expression vector encoding IB1/JIP1. After 24–36 h of transfection, cells were pre-treated for 1 h with BAPTA-AM (10 μM) prior to 20 h of ionophore treatment (1 μM). The 6-fold IB1/JIP1 degradation initiated by ionophore was totally prevented by BAPTA. BAPTA alone even stabilized IB1/JIP1 (Fig. 2).

These results indicate that the degradation mediated by the ionophore depends upon intracellular calcium concentration.

**Caspases Are Not Involved in Calcium-dependent IB1 Degradation**—Several protease families such as calpains and some caspases are calcium-dependent. Calpains are non-lysosomal cystein proteases that catalyze the endoproteolytic cleavage of specific substrates by a calcium-dependent process. As IB1/JIP1 degradation requires calcium, we first investigated whether calpains are the calcium-activated proteases responsible for IB1/JIP1 degradation. We pre-treated βTC-3 cells with...
a specific calpain inhibitor, the calpastatin peptide, before exposure to cytokines. Western blotting showed that a 24-h exposure to cytokines induced the preferential proteolysis of the M101 form of IB1/JIP1, which was not prevented by calpastatin. We also performed in vitro IB1/JIP1 digestion with recombinant calpain II. As predicted by the inhibition experiments with the calpastatin peptide, IB1/JIP1 was not cleaved by calpain II (data not shown). However, these in vitro digestions revealed a mobility shift in IB1/JIP1 migration in the presence of high calcium concentration.

High Calcium Concentration-induced IB1/JIP1 Degradation in βTC-3 Cellular Extracts—To confirm the correlation between IB1/JIP1 modification and high calcium concentrations, we performed in vitro dose-response experiments with cell extracts (called “in vitro calcium assay” in the following). Aliquots of βTC-3 cells extracts were incubated in the absence or presence of varying concentrations of calcium for 30 min at 30 °C. Increasing calcium concentration (0–6 mM) induced IB1/JIP1 modification: the IB1/JIP1 band detected by Western blot was shifted at 3 mM calcium (Fig. 4A) and at very high calcium concentration (6 mM), the shifted IB1/JIP1 disappeared, i.e. the amount of IB1/JIP1 in cell extracts was reduced by >95% (Fig. 4A). In the presence of EGTA, high calcium concentration had no effect on IB1/JIP1 (Fig. 4B). Calcium did not modulate β-tubulin stability (Fig. 4A), and the migration pattern of βTC-3 proteins remained unchanged after a high dose calcium treatment compared with untreated cell extracts (data not shown).

To verify the cation specificity in the IB1/JIP1 degradation, we tested the in vitro effect of high monovalent and divalent ion concentration on IB1/JIP1 stability. We did not observe any specific modification or degradation of IB1/JIP1 with any ions (K⁺, Na⁺, Mg²⁺, Mn²⁺, Ni²⁺, Zn²⁺) other than calcium (data not shown).

The Proteasome Is Involved in IB1/JIP1 Degradation Mediated by Calcium—The ubiquitin-proteolytic pathway is a major system for selective protein degradation in eukaryotic cells. One of the first steps in the process includes selective modification of lysine residues in the target protein by ubiquitination, which will cause a shift in the migration of the modified protein as observed with IB1/JIP1 (Fig. 4A). The ubiquitin residues target the protein for further degradation by the proteasome complex. We suspected that ubiquitination was the first calcium-dependent step in IB1/JIP1 modification. Because there is no ubiquitin ligase inhibitor available, we used lactacystin, a specific proteasome inhibitor, to block protein degradation. To determine whether ionophore-induced IB1/JIP1 degradation is mediated by the proteasome pathway, HeLa cells were transfected with an expression vector encoding IB1/JIP1. The HeLa cell line was used in this experiment, as lactacystin induced a high apoptotic rate in βTC-3 cells. After 24–36 h of transfection, cells were pre-treated for 1 h with 20 μM lactacystin, prior...
to 20 h of exposure to ionophore. To normalize the transfection rate, HeLa cells were cotransfected with a plasmid encoding the FLAG-tagged EGFP protein. The IB1/JIP1 content, determined by immunoblot analysis using anti-IB1 antibody, was decreased after 20 h of ionophore treatment, and lactacystin, determined by immunoblot analysis using anti-IB1 antibody, was used in the presence (5 mM) or absence of calcium and EGTA (10 mM) for 30 min at 30 °C.

Pull-down experiments provided further evidence for IB1/JIP1 ubiquitination. HeLa cells were transfected with FLAG-tagged IB1. Cell lysates were subjected to in vitro calcium assay, and the FLAG-IB1 was pulled down using an anti-FLAG resin. The immunoprecipitated FLAG-IB1 was subjected to anti-IB1 and anti-ubiquitin immunoblotting. Polyubiquitinated IB1 products were detected only in cells transfected with FLAG-IB1 but not in untransfected cells (Fig. 5B).

Taken together, these data strongly suggest that IB1/JIP1 is ubiquitinated and degraded by the ubiquitin-proteasome pathway.

**JNK Targets IB1/JIP1 for Ubiquitination**—Because ubiquitination targets IB1/JIP1 to efficient degradation by means of the proteasome pathway, we next investigated which mechanisms marked IB1/JIP1 for a calcium-dependent ubiquitination. IB1/JIP1, which tightly associates with JNK via JNK-binding domain, is phosphorylated at Thr-103 by JNK (21). It was previously demonstrated that the JNK substrates c-Jun as well as activating transcription factor-2, JunB, and p53 are responsible for JNK phosphorylation and/or for calcium-dependent ubiquitination. IB1/JIP1 stability was impaired when IB1/JIP1 was phosphorylated before the addition of high calcium concentration. In the presence of the JNKI1, IB1/JIP1 stability was increased at high calcium concentration (Fig. 6B).

To test the kinase specificity of IB1/JIP1 phosphorylation in vitro and modification of stability, we replaced JNK2 with ERK2 or cdc2/p34 kinases in in vitro calcium assays. The IB1/JIP1 mobility shift caused by phosphorylation by JNK2 was not observed after treatment with ERK2 or cdc2/p34 (Fig. 6C). In addition, no ubiquitination was found at high calcium concentration when IB1/JIP1 was incubated previously with ERK2 or cdc2/p34 (Fig. 6C).

**An Element from Amino Acid 328 to Amino Acid 437 Is Involved in the Ubiquitination Process of IB1/JIP1**—We observed that phosphorylation by JNK affected IB1/JIP1 stability at high calcium concentration in vitro. To localize a domain responsible for JNK phosphorylation and/or for calcium-dependent ubiquitination, we produced IB1/JIP1 deletion mutants. IB1/JIP1 mutants truncated at the C terminus to encode residues 1–281, 1–327, 1–438, 1–493, and 1–559 were generated by PCR coupled to a transcription/translation reaction. The truncated proteins were then phosphorylated by JNK2, which induced a mobility shift (Fig. 7) that is in agreement with the recent work of Nihalani et al. (21) showing IB1/JIP1 with IB1/JIP1, had no effect on IB1/JIP1 degradation. To assess whether phosphorylation affected IB1/JIP1 stability at high calcium concentration in vitro, we performed an in vitro calcium assay with non-phosphorylated and phosphorylated IB1/JIP1. As shown in Fig. 6B, IB1/JIP1 stability was impaired when IB1/JIP1 was phosphorylated before the addition of a high calcium concentration. In the presence of the JNKI1, IB1/JIP1 stability was increased at high calcium concentration (Fig. 6B).

An Element from Amino Acid 328 to Amino Acid 437 Is Involved in the Ubiquitination Process of IB1/JIP1—We observed that phosphorylation by JNK affected IB1/JIP1 stability at high calcium concentration in vitro. To localize a domain responsible for JNK phosphorylation and/or for calcium-dependent ubiquitination, we produced IB1/JIP1 deletion mutants. IB1/JIP1 mutants truncated at the C terminus to encode residues 1–281, 1–327, 1–438, 1–493, and 1–559 were generated by PCR coupled to a transcription/translation reaction. The truncated proteins were then phosphorylated by JNK2, which induced a mobility shift (Fig. 7) that is in agreement with the recent work of Nihalani et al. (21) showing IB1/JIP1
and exposed to high calcium concentrations. JIP1 proteins were produced by TNT, phosphorylated by active JNK2, calcium-dependent ubiquitination of IB1/JIP1.

Forms of IB1/JIP1 were already modified at 1.6 mM calcium concentration (Fig. 8). Both the mutated and truncated forms of IB1/JIP1 were incubated in the presence of increasing amounts of calcium for 30 min at 30 °C. The reaction was stopped by SDS-leading buffer, and the samples were analyzed by SDS-PAGE. We observed that a calcium-mediated shift of IB1/JIP1 was normally subjected to ubiquitination. These data strongly suggested that a domain localized between amino acid 328 and 437 sequence is involved in the calcium-dependent ubiquitination of IB1/JIP1. Truncated IB1/JIP1 proteins were produced by TNT, phosphorylated by active JNK2, and exposed to high calcium concentrations.

Phosphorylation at Thr-103. The phosphorylated form of the proteins was exposed to increasing calcium concentrations. We observed that amino acid 1–327 was insensitive to calcium-dependent modifications (Fig. 7). In contrast, amino acid 1–438 was normally subjected to ubiquitination. These data strongly suggested that a domain localized between amino acid 328 and amino acid 438 destabilizes IB1/JIP1 at high calcium concentrations and is necessary for IB1/JIP1 ubiquitination.

The S59N IB1 Mutation Destabilizes the Protein—The S59N point mutation in IB1/JIP1, linked to type 2 diabetes, was shown previously to increase the sensitivity of cells to IL-1β pro-apoptotic stimuli (17). To compare the stability of the wild-type IB1/JIP1 to the mutated S59N form and to the truncated N-terminal isoform (M101), HeLa cells were transfected with the expression vectors encoding the three proteins. Whole cell extracts were obtained, and aliquots were subjected to in vitro calcium assays. We observed that a calcium-mediated shift of the full-length wild-type IB1/JIP1 first occurred at 2 mM calcium concentration (Fig. 8). Both the mutated and truncated forms of IB1/JIP1 were already modified at 1.6 mM calcium concentration, which suggested that the N-terminal part of IB1/JIP1 stabilized the protein. Tubulin stability was not changed. The higher sensitivity of the M101 form to calcium-dependent ubiquitination correlates with the higher sensitivity of M101 to cytokines. Indeed, after a 24-h exposure to cytokines, only the lower band of IB1 was degraded (Fig. 3). Additional hours of exposure were necessary to observe the degradation of the upper band (Fig. 1A).

DISCUSSION

β-cell loss in type 1 diabetes mellitus seems to be essentially an apoptotic process initiated by the coordinate cytokine secretions of the immune cells surrounding the inflamed islets (26). The regulatory intracellular signaling network engaged by the binding of IL-1β, potentiating tumor necrosis factor-α, and IFN-γ to their receptors (which leads to apoptosis), represents a potential target for the development of novel therapeutic approaches. IB1/JIP1 is a promising tool for the prevention of β-cell loss.

IB1/JIP1, which is highly expressed in pancreatic β-cells, plays an anti-apoptotic function in insulin-producing cells by controlling the activity of the JNK signaling pathway (13). This control directly depends upon the amount of IB1/JIP1. The mechanisms that control the IB1 level in pancreatic β-cells are, therefore, potentially critical in deciding the cell-death or cell-survival response. Cytokines induce a marked reduction in the IB1/JIP1 content of β-cells (8). The basic mechanisms of the proteolytic degradation of IB1/JIP1 are unclear. In this study, we investigated the early events in the cytokine signaling pathway that can trigger the degradation of IB1/JIP1 and observed that intracellular calcium concentrations determine IB1/JIP1 stability. In the βTC-3 cell line, a calcium ionophore mimics the cytokine effect by inducing a decrease in IB1/JIP1 content and an increase in apoptotic rate. A correlation between cytokine concentration and intracellular calcium concentration was reported previously. Cytokines were found to induce a low voltage-activated calcium current in mouse βTC-3 cells and an increase in intracellular calcium in mouse islet cells that was associated with apoptosis (18). In addition, Calbindin-D28k, a cytosolic calcium-binding protein, was able to protect βTC-3 cells from cytokine-mediated apoptosis when overexpressed (27).

In crude β-cell extracts, addition of calcium is responsible for a massive migration shift of IB1/JIP1 on Western blots. At high calcium concentration, modified IB1/JIP1 is even completely degraded. The shift of IB1/JIP1 is clearly reminiscent of ubiquitin modification. The ubiquitin-proteolytic pathway is a ma-
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... system for selective protein degradation in eukaryotic cells. One of the first steps in the process includes selective modification of lysine residues in the corresponding protein by ubiquitination, which targets the protein for ubiquitin-dependent degradation by the proteasome complex. In βTC-3 cells in culture, we observed that lactacystin, a specific 20S proteasome inhibitor with no effect on cysteine and serine proteases, trypsin, and chymotrypsin (28) stabilized IB1/JIP1 after calcium ionophore treatment.

The experiments with lactacystin and BAPTA were conducted on HeLa cells, as both compounds induced a massive death process in βTC-3 cells. This high apoptotic rate in βTC-3 induced by BAPTA and lactacystin, two chemicals responsible for IB1/JIP1 stabilization (BAPTA and lactacystin) in HeLa cells, is not incompatible with the hypothesis that inhibition of IB1 degradation through the proteasome protects cells from apoptosis. Indeed, both BAPTA and lactacystin were reported to induce apoptosis in some cell types through signal transduction divergence from the JNK pathway. BAPTA was shown to promote apoptosis in MIN6 insulin-secreting cells (29), which are very sensitive to calcium homeostasis like other pancreatic β-cells. In MIN6 cells, BAPTA induces depletion of cytosolic and nuclear-free calcium concentrations, which is accompanied by an alteration of Bcl-2 to Bax expression ratio (mRNA and protein) leading to apoptosis (29). The causes of lactacystin-mediated cell death (30, 31) are unknown, but current reports have implicated accumulation of the tumor suppressor p53 (32, 33), heat-shock proteins (34), p27Kip1 (35), proapoptotic proteins (36), and Bid (37), or stabilization of active caspase-3 subunits (38) after proteasome blockade.

The signals that target proteins for ubiquitination are often unclear. In some cases, different patterns of phosphorylation or a partially conserved sequence motif are required. JNK targets its substrate for ubiquitination in a phosphorylation-dependent manner. Phosphorylated forms of the pro-apoptotic factors c-Jun and activating transcription factor-2 were found to be protected against JNK-targeted ubiquitination. As IB1/JIP1 is an anti-apoptotic substrate of JNK, we studied the role of JNK phosphorylation on its degradation. We observed that IB1/JIP1 phosphorylation by JNK is essential for its calcium-induced instability, implying that JNK participates actively in the regulation of IB1/JIP1 stability. After cytokine treatment and concomitant JNK activation, increased intracellular calcium induced ubiquitination of the scaffold.

To localize specific amino acid sequence motifs required for JNK- and calcium-dependent degradation, we produced C-terminus deletion in IB1/JIP1. We deduced that amino acid 328–437 contains element(s) responsible for the calcium-dependent ubiquitination. Examination of the amino acid sequences from amino acid 328 to 437 of IB1/JIP1 revealed two 9-amino acid consensus “destruction box” sequences originally described in cyclin molecules (EGALGXIXX) and required for its destruction by the ubiquitin-proteasome pathway (39, 40). Accordingly, we found RGSGLPPPG and RASLSDDTS motifs at positions 357–365 and 368–376, respectively.

Protein destruction by the ubiquitin-proteasome pathway is emerging as an important mechanism for the tight control of diverse cellular processes, including signal transduction from cell-surface receptors (41), gene transcription (42), angiogenesis (43), and cell-cycle progression (39). Aberrations in the proteolytic pathway are implicated in several disease states ranging from Alzheimer’s disease (44) to cancer (45). Our data suggest that the proteasome machinery, by controlling the IB1/JIP1 level in β-cells, could also be involved in the development of type 1 diabetes. Our results on the point mutation S59N at the N-terminus of IB1/JIP1, which has been associated with a familial form of type 2 diabetes (17), revealed that the proteasome could also be engaged in type 2 diabetes. Indeed, our results suggest that the decreased resistance to apoptosis after S59N mutation may be caused by sensitization of IB1/JIP1 to cytokine- and proteasome-dependent degradation. Our hypothesis that IB1/JIP1 degradation through the proteasome pathway might contribute to both types of diabetes (type 1 and 2) needs more investigation. However, increasing numbers of studies demonstrate direct relations between type 1 and type 2 diabetes, such as inflammatory mediators and islet β-cell failure (12, 46–51).

IB1/JIP1 acts as an anti-apoptotic protein, whose level seems to influence the β-cell death or survival response. Exposure to stress (e.g. by cytokines) induces a large down-regulation of IB1/JIP1 content, with a concomitant increased apoptotic rate (13). β-cells can be protected from stress-induced apoptosis by IB1/JIP1 overexpression (13) and by preventing IB1/JIP1 degradation. To learn how to protect IB1/JIP1 from degradation, we propose to determine which mechanisms are responsible for its down-regulation. In this study, we observed that IB1/JIP1 degradation is mediated by the ubiquitin-proteasome machinery. However, two preliminary events are required to prime IB1/JIP1 for ubiquitination: IB1/JIP1 has to be phosphorylated by JNK, and the intracellular calcium concentration has to be increased.

Further studies need to be performed to define more precisely the motif sequence of the destruction box that targets IB1/JIP1 for ubiquitination. This motif could be a good therapeutic target to delay IB1/JIP1 degradation and β-cell apopotosis.

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REFERENCES

1. Foulis, A. K., Liddle, C. N., Farquharson, M. A., Richmond, J. A., and Weir, R. S. (1986) Diabetes 29, 267–274
2. Hamaguchi, K., and Leiter, E. H. (1990) Diabetes 39, 415–425
3. Rabinovitch, A., Suarez-Franzon, W. L., Shi, Y., Morgan, A. R., and Bleackley, R. C. (1994) Diabetes 39, 733–738
4. Ishihashi, H., Hanaoka, T., Eguchi, Y., Nakajima, H., Miyagawa, J., Itoh, N., Tomita, K., Namba, M., Kawai, M., Nomura, T., Tsujimoto, Y., and Matsumura, Y. (1996) Diabetologia 39, 530–536
5. Dungan, A., Cunningham, J. M., Delaney, C. A., Lowe, J. E., Green, M. H., Bone, A. J., and Green, I. C. (1996) Diabetes 45, 183–189
6. Delaney, C. A., Pavlovic, D., Gros, A., Pipeleers, D. G., and Eizirik, D. L. (1997) Endocrinology 138, 2610–2614
7. Larsen, C. M., Wadst, K. A., Juhl, L. F., Andersen, H. U., Karlsen, A. E., Su, M. S., Seedorf, K., Shapiro, L., Dinarello, C. A., and Mandrup-Poulsen, T. (1998) J. Biol. Chem. 273, 15294–15300
8. Bonny, C., Nicol, P., and Waeger, G. (1998) J. Biol. Chem. 273, 1843–1846
9. Ammendrup, A., Maillard, A., Nielsen, K., Aabenh, A. N., Scrup, P., Dragshol, M. O., Mandrup-Poulsen, T., and Bonny, C. (2000) Diabetologia 43, 1468–1476
10. Negri, S., Oberson, A., Steinmann, M., Sauser, C., Nicol, P., Waeger, G., Schorderet, D. F., and Bonny, C. (2000) Genomics 64, 324–330
11. Bonny, C., Oberson, A., Negri, S., Sauser, C., and Schorderet, D. F. (2001) Diabetes 50, 77–82
12. Maedler, K., Segerer, P., Bis, F., Oberholzer, J., Joller-Jemelka, H. I., Spinas, G. A., Kaiser, N., Halban, P. A., and Donath, M. Y. (2002) J. Clin. Invest. 110, 851–860
13. Bonny, C., Oberson, A., Steinmann, M., Schorderet, D. F., Nicol, P., and Waeger, G. (2000) J. Biol. Chem. 275, 16466–16472
14. Whitmarsh, A. J., and Davis, R. J. (1998) Trends. Biochem. Sci. 23, 481–485
15. Gupta, S., Campbell, D., Derijard, B., and Davis, R. J. (1995) Science 267, 389–393
16. Dickens, M., Rogers, J. S., Cavanagh, J., Raitano, A., Xia, Z., Halpern, J. R., Greenberg, M. E., Sawyers, C. L., and Davis, R. J. (1997) Science 277, 655–656
17. Waeger, G., Delplanque, J., Bonny, C., Mooser, V., Steinmann, M., Widmann, C., Maillard, A., Miklosy, J., Dina, C., Hani, E. H., Vionnet, N., Nico, P., Routm, P., and Froqv, P. (2000) Nat. Genet. 24, 291–295
18. Wang, L., Bhattacharjee, A., Zuo, Z., Hu, F., Honkans, R. E., Berggren, P. O., and Li, M. (1999) Endocrinology 140, 1200–1204
19. Herrero, A., Van de Casteele, M., Kiopel, G., and Pipeleers, D. (1996) J. Clin. Invest. 98, 1568–1574
20. Kim, I. J., Lee, K. W., Park, B. Y., Lee, J. K., Park, J., Choi, I. Y., Eom, S. J., Chang, T. S., Kim, M. J., Yeom, Y. I., Chang, S. K., Lee, Y. D., Choi, E. J., and Han, P. I. (1999) J. Neurochem. 72, 1335–1343
21. Nihalani, D., Wong, H. N., and Holzman, L. B. (2003) J. Biol. Chem. 278, 26694–26702
Degradation of the JNK Scaffold Protein Islet-brain 1

22. Fuchs, S. Y., Dolan, L., Davis, R. J., and Ronai, Z. (1996) Oncogene 13, 1531–1535
23. Fuchs, S. Y., Xie, B., Adler, V., Fried, V. A., Davis, R. J., and Ronai, Z. (1997) J. Biol. Chem. 272, 32163–32168
24. Fuchs, S. Y., Adler, V., Buschmann, T., Yin, Z., Wu, X., Jones, S. N., and Ronai, Z. (1998) Genes Dev. 12, 2658–2663
25. Fuchs, S. Y., Tappin, I., and Ronai, Z. (2000) J. Biol. Chem. 275, 12560–12564
26. Eizirik, D. L., and Mandrup-Poulsen, T. (2001) Diabetologia 44, 2115–2133
27. Rabinovitch, A., Suarez-Pinzon, W. L., Sooy, K., Strynadka, K., and Christakos, S. (2001) Endocrinology 142, 3649–3655
28. Fenteany, G., Standaert, R. F., Lane, W. S., Choi, S., Corey, E. J., and Schreiber, S. L. (1995) Science 268, 726–731
29. Mizuno, N., Yoshitomi, H., Ishida, H., Kuromi, H., Kawaki, J., Seino, Y., and Seino, S. (1998) Endocrinology 139, 1429–1439
30. Shah, S. A., Potter, M. W., and Callery, M. P. (2001) Surg. Oncol. 10, 43–52
31. Almond, J. B., and Cohen, G. M. (2002) Leukemia 16, 433–443
32. Lopes, U. G., Yao, R., and Cooper, G. M. (1997) J. Biol. Chem. 272, 12893–12896
33. Wagenknecht, B., Hermisson, M., Eitel, K., and Weller, M. (1999) Cell Physiol. Biochem. 9, 117–125
34. Buhl, K. T., Goldberg, A. L., and Nigam, S. K. (1997) J. Biol. Chem. 272, 9086–9092
35. Drexler, H. C. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 855–860
36. Li, B., and Deu, Q. P. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 3850–3855
37. Breitschopf, K., Zeiher, A. M., and Dimmeler, S. (2000) J. Biol. Chem. 275, 21648–21652
38. Chen, L., Smith, L., Wang, Z., and Smith, J. B. (2003) Mol. Pharmacol. 64, 334–345
39. Glotzer, M., Murray, A. W., and Kirschner, M. W. (1991) Nature 349, 132–138
40. King, R. W., Glotzer, M., and Kirschner, M. W. (1996) Mol. Biol. Cell 7, 1343–1357
41. Mimnaugh, E. G., Chavany, C., and Neckers, L. (1996) J. Biol. Chem. 271, 22796–22801
42. Treier, M., Staszewski, L. M., and Bohmann, D. (1994) Cell 78, 787–798
43. Okawa, T., Sasaki, T., Nakamura, M., Shimamura, M., Tanahashi, N., Omura, S., and Tanaka, K. (1998) Biochem. Biophys. Res. Commun. 246, 243–248
44. Kim, T. W., Pettingell, W. H., Hallmark, O. G., Morr, R. D., Wasco, W., and Tann, R. E. (1997) J. Biol. Chem. 272, 11006–11010
45. Spataro, V., Norbury, C., and Harris, A. L. (1998) Br. J. Cancer 77, 448–455
46. Donath, M. Y., Stirling, J., Maedler, K., and Mandrup-Poulsen, T. (2003) J. Mol. Med. 81, 455–470
47. Mathis, D., Vence, L., and Benoist, C. (2001) Nature 414, 792–798
48. Pietropaolo, M., Barinas-Mitchell, E., Pietropaolo, S. L., Kuller, L. H., and Trucce, M. (2000) Diabetes 49, 32–38
49. Bowley, M. J., Mackay, I. R., Chen, Q. Y., Knowles, W. J., and Zimmet, P. Z. (1992) Diabetes 41, 548–551
50. Wilkin, T. J. (2001) Diabetologia 44, 914–922
51. Maedler, K., Spinas, G. A., Lehmann, R., Berger, P., Weber, M., Fontana, A., Kaiser, N., and Donath, M. Y. (2001) Diabetes 50, 1683–1690
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