Chapter 6
CREB Glycosylation Potentiates Apoptosis in Pancreatic Cell Lines by Suppressing Gene Expression of Targets of CREB Regulation

Summary. CREB regulates cell specific processes such as insulin secretion, growth and survival in pancreatic β-cells [1-8]. The survival of pancreatic β-cells depends on the balance between survival and apoptotic signaling cascades that antagonize one another (Fig. 6-1). The fate of the cell depends on the outcome of the "tug of war" between these two forces [9]. Three kinase signaling cascades that promote β-cell survival are the cAMP-dependent protein kinase (PKA), phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) and mitogen activated protein kinase kinase (MEK)/extracellular signal related kinase (ERK) cascades [4, 10, 11]. The effects of all three kinases signaling cascades are, in part, mediated by activation of CREB. Of particular interest to us were studies showing that CREB phosphorylation by PKA and MEK/ERK pathways leads to expression of insulin receptor substrate 2 (IRS2), a gene critical for β-cell survival [4, 12-14]. We have looked at the effect that *O*-GlcNAc glycosylation has on CREB activity and the outcomes of increased *O*-GlcNAc glycosylation on pancreatic β-cell survival. We found that the inhibition of CREB activity by *O*-GlcNAc glycosylation results in a decrease in the CREB-dependent expression of IRS2, as well as other targets of CREB, and a subsequent increase in the level of programmed cell death observed in these cells.

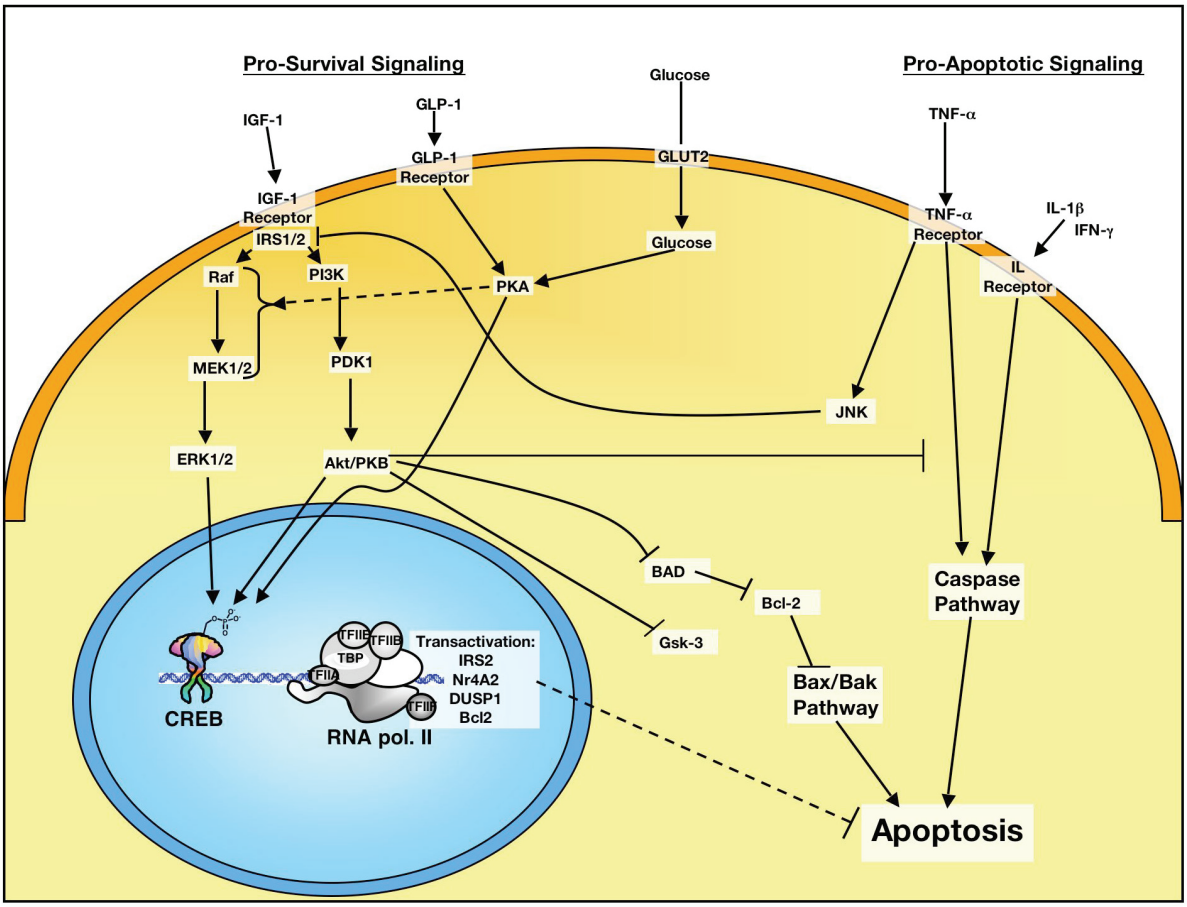

Figure 6-1. Survival and apoptotic signaling in pancreatic β-cells: the role for insulin receptor substrates and CREB. β-cell survival is supported by activation of CREB via PKA,MEK/ERK and PI3K/Akt kinase signaling pathways. Both MEK/ERK and PI3K/Akt pathways are activated by insulin/IGF-1 receptors that are dependent on IRS2 to transduce the pro-survival signal. PKA is activated by glucagon-like peptide 1 (GLP-1) and moderate elevations in glucose that can directly phosphorylate CREB and induce the MEK/ERK signaling pathway. In a positive feedback loop CREB regulates the transcription of the IRS2, which itself is required for insulin/IGF-1 signaling. CREB has also been shown to express a number of other genes involved in pro-survival signaling, including Nr4A2, DUSP1 and Bcl-2 and Bcl-2 associated proteins. In opposition to these pathways are cytokine receptor pathways such as TNFα. TNFα leads to apoptosis by activating the caspase pathway and activation of Jun N-terminal Kinase (JNK), which attenuates insulin/IGF-1 signaling by Ser/Thr phosphorylation of IRS1 and 2 [1-11].

Glucosamine Treatment Reduces Expression of IRS2 and Other CREB-Dependent Genes. The ability of CREB to regulate pancreatic β-cell survival led us to examine whether CREB glycosylation leads to silencing of IRS2. CREB has been shown to promote islet cell proliferation and viability by regulating the expression of cell cycle

associated factors, cyclin A and anti-apoptotic factors such as cIAP-2, IRS2 and Bcl-2 [4, 13, 15, 16].

We first tested whether activation of the PKA, which has been shown to induce CREB-dependent expression of the luciferase reporter, can also induce expression of endogenous targets of CREB-dependent gene expression. Observation of IRS2 protein levels showed that forskolin (Fsk) treatment increased IRS2 expression, consistent with previous studies, and that pretreatment with GlcN reduced Fsk-induced expression of IRS2 by approximately 21% (Fig. 6-2) [4]. At the same time that decreases in the levels of IRS2 were observed, there was no significant change in the level of CREB present in the cells (Fig. 6-2). Thus the decrease in IRS2 levels are likely as a result of increased protein degradation or a reduction CREB transactivation of the *IRS2* gene.

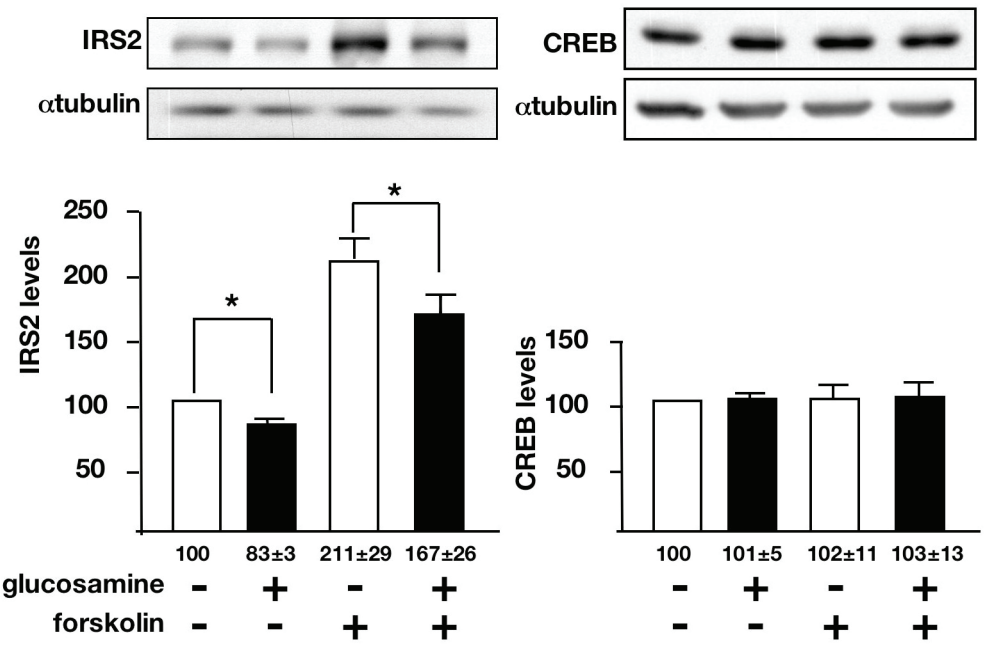

Figure 6-2. GlcN treatment of pancreatic HIT-T15 cells reduces basal and Fsk-induced IRS2 levels but has no effect on CREB. Cells were treated in the presence or absence of GlcN (10 mM, 9 h). Fsk (10 μM) was added to the medium 3 h after addition of GlcN, and the cells were incubated for an additional 6 h. IRS2 protein expression was analyzed by immunoblotting of cell lysates using anti-IRS2 (Upstate, Charlottesville, VA; 1:1,000) and anti-CREB (Upstate, Charlottesville, VA; 1:1,000) antibodies and corrected to protein concentration as measured by anti-αtubulin (Sigma-Aldrich, St. Louis, MO; 1:5000). Values were normalized relative to the untreated cells and represent the mean ± SEM (Student's T-test, * $P < 0.03$; n = 4).

Previous reports have found that *O*-GlcNAc glycosylation is capable of affecting protein levels at the level of transcription, translation and degradation [17, 18]. To confirm that IRS2 expression was affected at the level of transcription, relative *IRS2* mRNA levels were measured 30 min after Fsk treatment by RT-PCR. *IRS2* gene transcription was induced by Fsk treatment and the expression was inhibited by GlcN (Fig. 6-3). In contrast, transcription of IRS1, which along with IRS2 is responsible for transduction of insulin and IGF-1 receptor signaling [19], was unaffected by Fsk activation of cAMP signaling and showed only a slight inhibition in response to GlcN treatment (Fig. 6-3B). The promoter of *IRS1* does not contain any full or half-CRE sites and previous studies have shown that the *IRS1* gene is not controlled by CREB [4, 20].

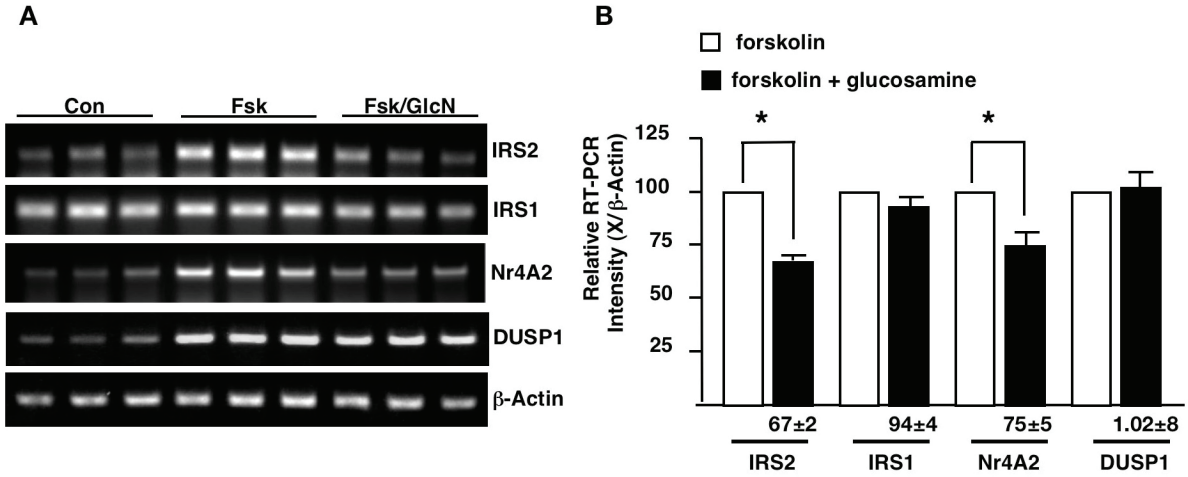

Figure 6-3. CREB-dependent gene expression is suppressed by glucosamine in HIT-T15 cells. Fsk-induced transcription of CREB-target genes, IRS2 and NR4A2, are specifically inhibited by GlcN treatment. **(A)** HIT-T15 β-cells were incubated in the presence or absence of GlcN (10 mM) 3 h prior to the addition of Fsk (10 μM). 60 min after the addition of Fsk, RNA was harvested from cells and used in first strand cDNA synthesis using oligo $dT_{12\text{-}18}$ primers (Invitrogen). cDNA was used in semi-quantitative PCR to determine the relative levels of *IRS1*, *IRS2*, DUSP1 and NR4A2 transcripts normalized to level of β-actin transcripts. **(B)** GlcN reduced *IRS2* and *Nr4A2* expression on an order equivalent to that observed by IRS2 immunoblotting shown in Figure 6-2. *DUSP1*, which is also controlled by CREB, was not affected by GlcN. The relative intensity of PCR products was quantified with an Alpha Imager 3400 (Alpha Innotech Corp., San Leandro CA) using software AlphaEaseFC software version 4.0.1 (Alpha Innotech Corp.). Values represent the mean ± SEM (Student's T-test, * $P < 0.04$; n = 5).

IRS1 is thought to play a larger role in the transduction of insulin signaling and, unlike IRS2, knockout of IRS1 has a minimal effect on pancreatic β-cell survival [19].

In addition to IRS2 transcription, we looked at the effects of GlcN on the transcription of other targets of CREB activity. Gene-targets were chosen based on the reported CREB:DNA occupancy reported in genome-wide ChIP studies [20]. Transcription of the steroid nuclear hormone receptor NR4A2 and the dual-specificity phosphatase 1 (DUSP1, also known as MAPK kinase phosphatase 1), both identified as targets of CREB, was induced robustly by Fsk treatment (Fig. 6-3A) [4, 20, 21]. The Fsk-induced transcription of Nr4A2 was inhibited by GlcN (Fig. 6-3B). NR4A2, like IRS2, has been implicated in the prevention of apoptosis, and the reduction of Nr4A2-induced expression by GlcN could reduce a cell's ability to withstand increases in pro-apoptotic stress [4, 21-23]. Surprisingly, no changes in the level of Fsk-induced transcription of DUSP1 were observed in response to GlcN treatment (Fig. 6-3B). DUSP1, like IRS2 and Nr4A2, promotes cell survival [24]. DUSP1 inhibits apoptotic pathways through dephosphorylation/deactivation of p38 MAPK and c-Jun NH_2-terminal kinase (JNK) (Fig. 6-1) [25, 26]. In fact, DUSP1 over expression has been linked to pancreatic cancer and may contribute to tumorgenicity through suppression of MAPK and JNK signaling [27]. It may be that *DUSP1* expression is also abnormally expressed in the pancreatic β-cell lines HIT-T15 and MIN6, used in these studies.

These findings support the role of CREB in the regulation of anti-apoptotic genes *IRS2*, *NR4A2* and *DUSP1* and suggest that glycosylation of CREB can reduce the level of cAMP-induced expression of *IRS2* and *Nr4A2*. The different effect of GlcN treatment on IRS2 and Nr4A2 compared with DUSP1 suggests that *O*-GlcNAc glycosylation may

enable differential regulation of specific genes and may depend on the occupancy of OGT at particular promoters or require additional cofactors to mediate the down-regulation of CREB-target gene expression.

OGT Associates Directly With RNA Polymerase II-Dependent Gene Promoters. A large number of proteins involved in gene expression are *O*-GlcNAc glycosylated (Table 2-1), and the fact that OGT is concentrated in the nucleus suggests an active role for OGT in the regulation of gene expression. Studies have found that OGT can interact with a number of co-repressor complexes, and these interactions would have the potential to confer specificity to OGT activity through directed localization of OGT. Chromatin immunoprecipitation using an anti-OGT antibody was used to investigate whether OGT associates specifically with CREB-regulated genes.

IRS1, *IRS2* and *NR4A2* promoter DNA were all recovered by chromatin immunoprecipitation (ChIP) using an OGT-specific antibody in MIN6 cells and pancreatic islets, whereas only *IRS2* and *NR4A2* promoter DNA were isolated by ChIP using a CREB antibody (Fig. 6-4A). These findings suggest that the *IRS2* and *NR4A2* genes are direct targets for regulation by OGT and CREB in pancreatic β-cells. Interestingly, DUSP1 was isolated by both CREB and OGT-ChIP experiments in isolated pancreatic islets but was only isolated by CREB-ChIP experiments in MIN6 cells (Fig. 6-4A). This difference could reflect changes that result from the immortalization of the pancreatic β-cell lines, as noted earlier dysfunction in the regulation of DUSP1 expression is linked to pancreatic cancer cell tumorigenicity [27, 28].

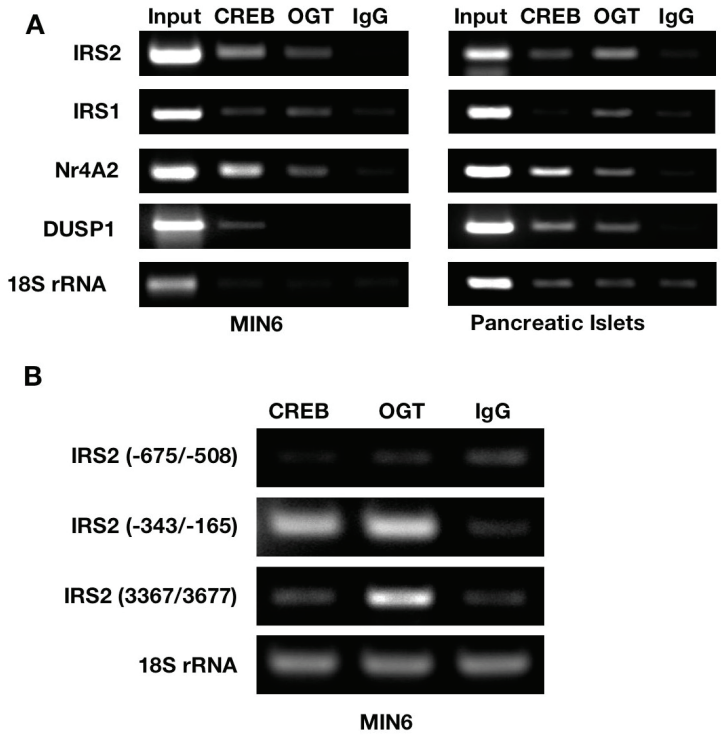

Figure 6-4. OGT is associated strongly with CRE-dependent promoters and RNA pol. II-dependent genes. Chromatin and associated proteins were cross-linked in MIN6 β-cells and pancreatic islets with 1% formaldehyde for 20 min. Chromatin was sheared by sonication to average length of 500 bp, followed by immunoprecipitation with anti-CREB, anti-OGT or control (mouse IgG) antibodies. The DNA extracted from the respective immunoprecipitates was amplified using primers proximal to the half-CRE site in the promoter region of IRS2 (-675/-508, -343/-165 and 3367/3677), bracketing the full-CRE in the promoter of *NR4A2* (-2677/–2521), bracketing the full-CRE in the promoter of *DUSP1* (-336/-94), within the promoter of *IRS1* (-587/–465) and the control gene 18S rRNA (+878/+1096; the translational start site is designated s bp=1). **(A)** Chromatin immunoprecipitation analysis indicates that CREB and OGT are recruited to the *IRS2* and *NR4A2* promoters. **(B)** CREB and OGT bind to the IRS2 promoter with varying degrees of specificity. Gel images are representative of 4 independent experiments.

When multiple primer sets were used to test the specificity of the CREB and OGT interaction with the *IRS2* gene, ChIP analysis showed that CREB was bound precisely to a region of the promoter roughly 200 bp upstream of the initial ATG. While OGT was enriched within the promoter region of *IRS2*, it was also bound to DNA further downstream, well within the coding region, suggesting a less stringent means of localization for OGT and associated corepressors (Fig. 6-4B). Similar experiments have been performed on a genome-wide scale to identify transcription factor, such as CREB,

and other DNA-binding factor localization and specific targeting of a transcription factor within a gene [20, 29-32].

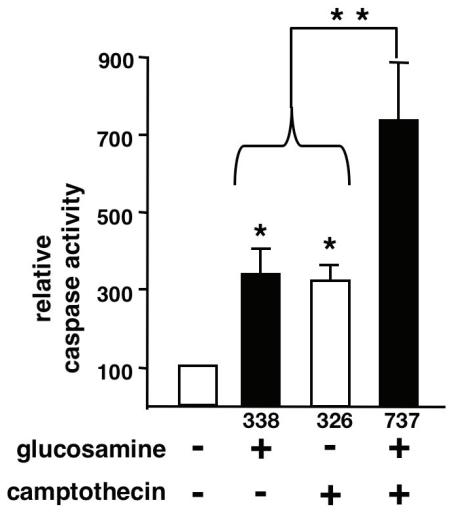

Figure 6-5. *O*-GlcNAc glycosylation of CREB attenuates β-cell survival. GlcN treatment of pancreatic MIN-6 β-cells induces apoptotic cell death. Cells were treated with GlcN (10 mM) and/or camptothecin, which induces apoptosis via DNA damage, (0.2 μg/mL) for 18 h, and apoptosis was quantified by measuring caspase-3/7 activity (Promega, Madison, WI). Values were normalized relative to the untreated GlcN control and represent the mean ± SEM (Student's T-test, * and **P < 0.05; n = 4).

Glucosamine Stimulates Apoptotic Signaling. IRS2 has been shown to promote islet-cell survival through a cAMP-dependent pathway that potentiates insulin/IGF-1 signaling through the phosphatidylinositol 3-kinase (PI3K)/Akt kinase and ERK1/2 signaling pathways [5, 11, 33]. Consistent with a role for IRS2 in β-cell survival, the addition of GlcN promoted β-cell apoptosis, as demonstrated by enhanced caspase-3/7 activity (Fig. 6-5). GlcN-induced apoptosis is of the same magnitude as that produced by camptothecin (0.2 mg/ml), which causes apoptosis via irreversible DNA-double strand breaks during replication and transcription [34]. Co-treatment of cells with GlcN and camptothecin showed that activation of caspase-3/7 by GlcN and camptothecin was additive. To examine the contribution of the *O*-GlcNAc modification to the apoptotic effects of GlcN, we used small interfering RNA (siRNA) to silence the expression of OGT. Introduction of OGT siRNA into MIN6 cells produced a significant reduction in OGT expression (Fig. 6-6). Cells transfected with the scramble siRNA control displayed increased caspase-3/7

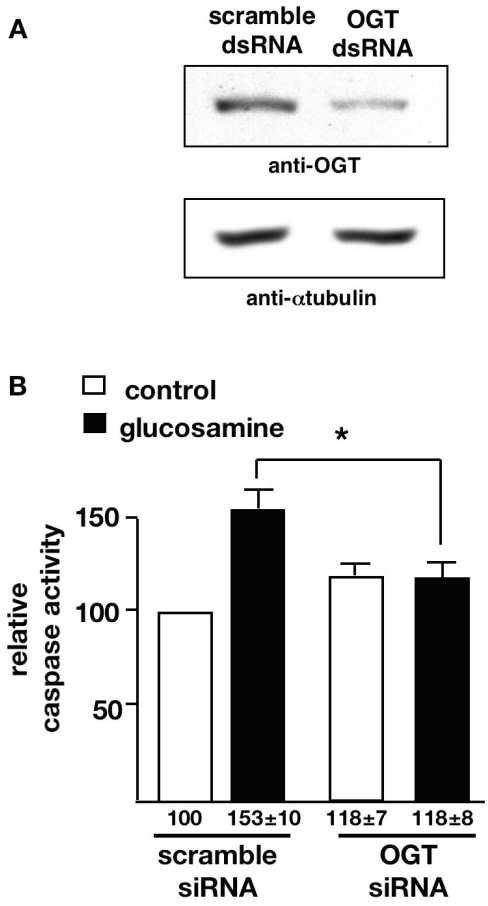

Figure 6-6. Silencing of OGT expression prevents GlcN-induced apoptosis in MIN6 cells. **(A)** Transfection of MIN6 cells with OGT siRNA, but not the scramble siRNA control, significantly reduces OGT expression and global *O*-GlcNAc glycosylation levels. Cells were transfected with a dsRNA (500 ng/mL) by electroporation (Amaxa). After 48 h, the cell lysates were analyzed by immunoblotting with an anti-OGT (Sigma-Aldrich, 1:1,000 dilution) or anti-αtubulin antibody (Sigma-Aldrich, 1:5,000). **(B)** Silencing of OGT using siRNA blocks GlcN-induced cell death. MIN-6 cells were transfected with OGT siRNA or scrambled siRNA as a control 30 h prior to stimulation with GlcN (10 mM, 18 h). Relative caspase-3/7 activity was measured using a fluorometric caspase-3/7 assay (Promega, Madison, WI). Values were normalized relative to untreated cells and represent the mean ± SEM. (Student's T-test, * $P < 0.05$; n = 14).

activity in response to treatment with GlcN and Fsk (Fig. 6-6). In contrast, no change in GlcN-induced apoptosis was observed upon reduction of OGT expression. The relative induction of caspase-3/7 activity, caused by GlcN treatment, observed in cells treated with the scrambled siRNA was muted when compared to the levels previously observed (Fig. 6-5). It may be the case that the electroporation method used to shuttle the siRNA into the cell also increases caspase-3/7 activity and as a result reduces the magnitude of the GlcN-induced apoptosis. The observation that introduction of OGT siRNA into β-cells increases the overall level of apoptosis is consistent with previous studies, which demonstrate that OGT is required for cell survival and that hypo-glycosylation may also be deleterious to cell survival [35]. We suggest that the functions of OGT may be finely balanced, such that under normal conditions OGT contributes to the survival of

β-cells, but conditions leading to enhanced OGT activity can have deleterious effects through a mechanism involving the glycosylation of CREB.

Finally, we tested whether blocking CREB glycosylation would overcome the apoptotic effects of GlcN. Cells transiently expressing either wild-type CREB or the AAA mutant form were monitored for caspase activity following treatment with GlcN and Fsk. Cells were monitored to ensure that expression of recombinant CREB did not lead to transcriptional squelching of CREB activity (Fig. 5-11), a phenomena where in over expression of a transcription activator inhibits the activators activity, presumably by competition for cofactor and RNA polymerase II constituents between the DNA bound factor and an excess unbound activator [36]. While GlcN treatment led to increased caspase-3/7 of pancreatic β-cells expressing wild-type CREB, prevention of glycosylation via the AAA mutation reduced the level of caspase-3/7 activity (Fig. 6-7). These results provide direct evidence that *O*-

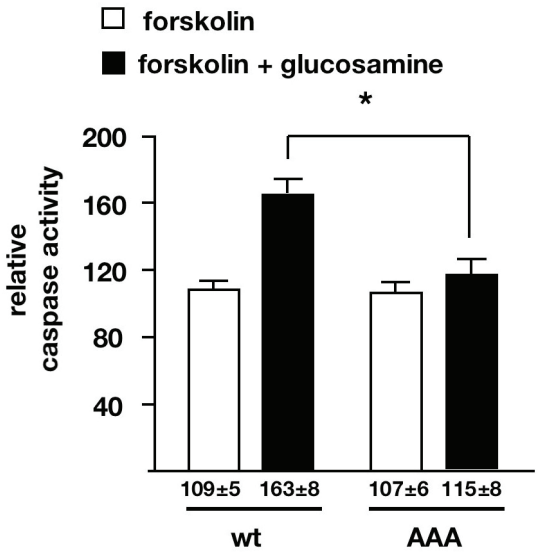

Figure 6-7. Blocking CREB glycosylation rescues β-cells from GlcN-induced cell death. HIT-T15 cells were transfected with expression vectors encoding either wild-type CREB or a triple-alanine mutant form, in which the glycosylation sites were converted to alanines. After 24 h, cells were treated when indicated with GlcN (10 mM, 18 h) and/or Fsk (10 μM, 6 h). Relative caspase-3/7 activity was measured as described in Figure 6-5. Values were normalized relative to Fsk-treated cells and represent the mean ± SEM (Student's T-test, * P <0.01; n =4).

GlcNAc glycosylation of CREB contributes to GlcN-induced pancreatic β-cell death and that inhibition of CREB glycosylation may play a protective role.

Conclusions

We have demonstrated that *O*-GlcNAc glycosylation of CREB represses CREB-dependent transcription in pancreatic cells. Glycosylation functionally opposes phosphorylation and provides a novel mechanism for the regulation of CREB activity. One important consequence of CREB glycosylation may be to down-regulate the *IRS2* gene, thereby reducing the strength of insulin and IGF-1 anti-apoptotic signaling and thus promoting the death of pancreatic β-cells (Fig. 6-8). We also observed a reduction in the expression of the *NR4A2* gene in response to GlcN treatment of pancreatic cells, but no effect on the DUSP1 gene, which is also controlled by CREB. These observations suggest that *O*-GlcNAc glycosylation may enable CREB to regulate different programs of gene expression by moderating the effect of CREB phosphorylation through the targeting of OGT to specific CREB-bound promoters.

Studies have shown that OGT associates with the co-repressors mSin3a, N-CoR and SMRT, suggesting that OGT may play a role in creating a silencing environment through histone deacetylation and *O*-GlcNAc glycosylation [37, 38]. The make-up of repressor complexes with which OGT is associated may dictate its targeting. As OGT is recruited directly to the *NR4A2*, *IRS1* and *IRS2* promoters, the targeting of OGT and *O*-GlcNAc modifications to particular promoter regions might provide a mechanism to silence specific genes in response to increased flux through the hexosamine biosynthesis pathway and other stimuli. In the future it would be interesting to undertake comprehensive study of the targeting of OGT to promoters and its effects on CREB-

dependent gene expression in order to determine which select programs of CREB-dependent gene expression are targeted by OGT, or whether that *O*-GlcNAc glycosylation affects the entire CREB regulon.

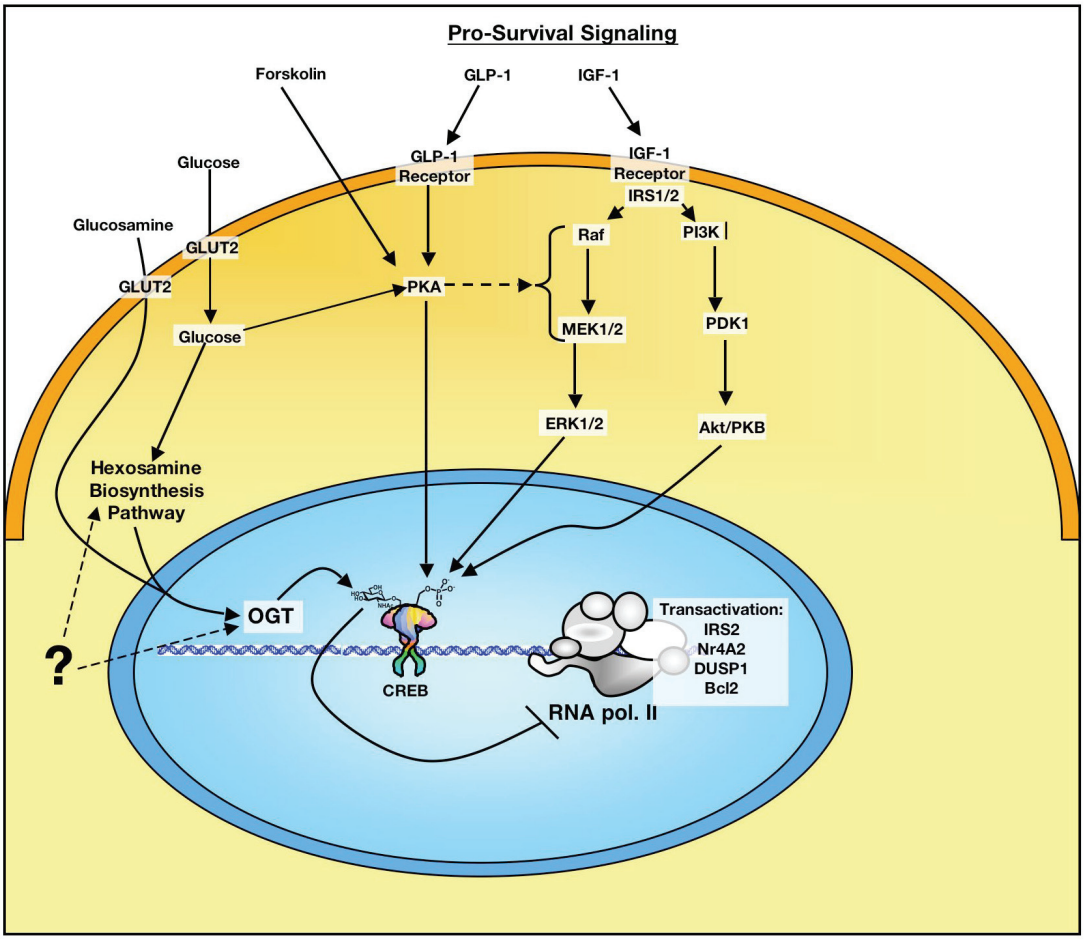

Figure 6-8. CREB glycosylation and the regulation of β-cell survival. Increasing CREB glycosylation via GlcN treatment moderates the phosphoSer133-dependent CREB transactivation by forskolin. OGT associates directly with CREB at promoters to inhibit CREB-dependent gene expression. This inhibition may result in an increase in apoptosis as the expression of CREB-dependent anti-apoptotic genes (*IRS2* and *Nr4A2*) are reduced.

Role for CREB Glycosylation Beyond Pancreatic β-Cells. CREB-dependent gene expression is important in a number of cell types, including neurons, where it is activated in response to the consolidation of long-term memory as well as pro-survival signaling. In neurons, as in pancreatic β-cells, the insulin/IGF-1 signaling pathway has been

implicated in the inhibition of apoptosis, and both cell types highly express OGT [39-41]. Given the importance of CREB and insulin/IGF-1 signaling in neuronal cell survival and the prevalence of OGT in both cell types, future experiments could address the possibility that OGT acts in a similar manner in neurons to moderate CREB-dependent gene expression and enhance cellular apoptosis (Fig. 6-9).

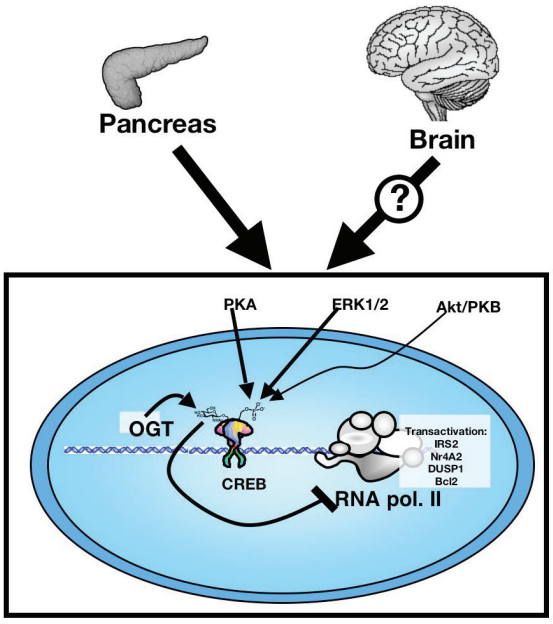

Figure 6-9. Role of CREB glycosylation in cell survival and possible alternative functions. We have shown that *O*-GlcNAc glycosylation can attenuate CREB-mediated pro-survival signaling in pancreatic β-cells. Similar pro-survival pathways are integral in neuronal cell types as well. Future investigations should determine whether glycosylation of CREB plays a role specific to the pancreas or is also involved in the regulation of cell survival in other cell types.

Regulation of gene expression involves complex interactions among histones, transcription factors, coactivators and corepressors. An emerging concept is that transcription factors may be modified by multiple post-translational modifications simultaneously, coordinating distinct biological programs. Our studies demonstrate for the first time that *O*-GlcNAc glycosylation of CREB modulates its ability to activate gene expression. The levels of global *O*-GlcNAc glycosylation reflect the net effect of metabolic flux through the hexosamine biosynthesis pathway, and may thus function as a new integrating signal to coordinately regulate distinct patterns of gene expression.

Bibliography

1. Goodison, S., S. Kenna, and S.J. Ashcroft, *Control of insulin gene expression by glucose*. Biochemical Journal, 1992. **285**: p. 563-568.

2. Hussain, M.A., P.B. Daniel, and J.F. Habener, *Glucagon stimulates expression of the inducible cAMP early repressor and suppresses insulin gene expression in pancreatic beta-cells*. Diabetes, 2000. **49**(10): p. 1681-1690.

3. Melloul, D., S. Marshak, and E. Cerasi, *Regulation of insulin gene transcription*. Diabetologia, 2004. **45**(3): p. 309-326.

4. Jhala, U.S., et al., *cAMP promotes pancreatic beta-cell survival via CREB-mediated induction of IRS2*. Genes and Development, 2003. **17**: p. 1575-1580.

5. Tuttle, R.L., et al., *Regulation of pancreatic beta-cell growth and survival by the serine/threonine protein kinase Akt1/PKBalpha*. Nature Medicine, 2001. **7**(10): p. 1133-1137.

6. Ueki, K., et al., *Total insulin and IGF-1 resistance in pancreatic beta-cell causes in diabetes*. Nature Genetics, 2006. **38**(5): p. 583-588.

7. Taniguichi, C.M., B. Emanuelli, and C.R. Kahn, *Critical nodes in signaling pathways: insights into insulin action*. Nature Reviews, 2006. **7**: p. 85-95.

8. Heit, J.J., S.K. Karnik, and S.K. Kim, *Intrinsic regulators of pancreatic beta-cell proliferation*. Annual Review of Cellular Developmental Biology, 2006. **22**: p. 311-338.

9. Mandrup-Poulsen, T., *beta Cell Apoptosis*. Diabetes, 2001. **50**: p. S58-S63.

10. Dickson, L.M. and C.J. Rhodes, *Pancreatic beta-cell growth and survival in the onset of type 2 diabetes: a role for protein kinase B in the Akt?* American Journal of Endocironlogy and Metabolism, 2004. **287**: p. E192-E198.

11. Liu, W., et al., *Activation of Phosphatidylinositol 3-Kinase Contributes to Insulin-Like Growth Factor I-Mediated Inhibition of Pancreatic ß-Cell Death*. Endocrinology, 2002. **143**(10): p. 3802-3812.

12. Costes, S., et al., *ERK1/2 control phosphorylation and protein level of cAMP-responsive element-binding protein*. Diabetes, 2006. **55**: p. 2220-2223.

13. Nishihara, H., et al., *Cyclic AMP promotes cAMP-responsive element binding protein-dependent induction of cellular inhibitor of apoptosis protein-2 and suppresses apoptosis of colon cancer cells through ERK1/2 and p38 MAPK*. The Journal of Biological Chemistry, 2004. **279**(25): p. 26176-26183.

14. Park, S., et al., *Exendin-4 uses Irs2 signaling to mediate pancreatic beta-cell growth and function*. The Journal of Biological Chemistry, 2006. **281**(2): p. 1159-1168.

15. Inada, A., et al., *Overexpression of Inducible Cyclic AMP Early Repressor Inhibits Transactivation of Genes and Cell Proliferation in Pancreatic beta*. Molecular and Cellular Biology, 2004. **24**(7): p. 2831-2841.

16. Hui, H., et al., *Glucagon-Like Peptide-1 Inhibits Apoptosis of Insulin-Secreting Cells via a Cyclic 5'-Adenosine Monophosphate-Dependent Protein Kinase A- and a Phosphatidylinositol 3-Kinase-Dependent Pathway*. Endocrinology, 2003. **144**(4): p. 1444-1455.

17. Datta, B., et al., *Glycosylation of Eukaryotic Peptide Chain Initiation Factor 2 (eIF-2)- associated 67-kDa Polypeptide (p"') and Its Possible Role in the Inhibition of eIF-2 Kinase-catalyzed Phosphorylation of the eIF-2 a-Subunit*. The Journal of Biological Chemistry, 1989. **264**(34): p. 20620-20624.

18. Zhang, F., et al., *O-GlcNAc Modification is an Endogenous Inhibitor of the proteasome*. Cell, 2003. **115**: p. 715-725.

19. Burks, D.J. and M.F. White, *IRS proteins and beta-cell function*. Diabetes, 2001. **50**: p. S140-S145.

20. Zhang, X., et al., *Genome-wide analysis of cAMP-response element binding protein occupancy, phosphorylation, and target gene activation in human tissues*. Proceedings of the National Academy of Sciences of the United States of America, 2005. **102**: p. 4459-4464.

21. Fass, D.M., J.E. Butler, and R.H. Goodman, *Deacetylase activity is required for cAMP activation of a subset of CREB target genes*. The Journal of Biological Chemistry, 2003. **278**(44): p. 43014-43019.

22. Ke, N., et al., *Nuclear Hormone Receptor NR4A2 is Involved in Cell Transformation and Apoptosis*. Cancer Research, 2004. **64**: p. 8208-8212.

23. Li, Q.-X., et al., *NR4A1,2,3-an orphan nuclear hormone receptor family involved in cell apoptosis and carcinogenesis*. Histology and Histopathology, 2006. **21**: p. 533-540.

24. Wu, J.J. and A.M. Bennett, *Essential role for mitogen-activated protein (MAP) kinase phosphatase-1 in stress-responsive MAP Kinase and cell survival signaling*. The Journal of Biological Chemistry, 2005. **280**(16): p. 16461-16466.

25. Chi, H., et al., *Dynamic regulation of pro- and anti-inflammatory cytokines by MAPK phosphatase I (MKP-1) in innate immune responses*. Proceedings of the National Academy of Sciences of the United States of America, 2006. **103**(7): p. 2274-2279.

26. Zhang, B., et al., *Parathyroid hormone related protein induces insulin expression through activation of MAPK-specific phosphatase-1 that dephosphorylates c-Jun NH2-terminal kinase in pancreatic beta-cells*. Diabetes, 2003. **52**: p. 2720-2730.

27. Liao, W., et al., *Down-regulation of the dual-specificity phosphatase MKP--1 suppresses tumorigenicity of pancreatic cancer cells*. Gastroenterology, 2003. **124**: p. 1830-1845.

28. Lin, Y.-W. and J.-L. Yang, *Cooperation of ERK and SCFSkp2 for MKP-1 destruction provides a positive feedback regulation of proliferating signaling*. The Journal of Biological Chemistry, 2006. **281**(2): p. 915-916.

29. Farris, S.D., et al., *Transcription-induced Chromatin Remodeling at the c-myc Gene Involves the Local Exchange of Histone H2A.Z*. The Journal of Biological Chemistry, 2005. **280**(26): p. 25298-25303.

30. Liang, G., et al., *Distinct localization of histone H3 acetylation and H3-K4 methylation to the transcription start sites in the human genome*. Proceedings of the National Academy of Sciences of the United States of America, 2004. **101**(19): p. 7357-7362.

31. Schlisio, S., et al., The EMBO Journal, 2002. **21**(21): p. 5775-5786.

32. Moqtaderi, Z. and K. Struhl, *Genome-wide occupancy profile of the RNA polymerase II machinery in Saccromyces cerevisiae reveals loci with incomplete transcription complexes*. Molecular and Cellular Biology, 2004. **24**: p. 4118-4127.

33. Withers, D.J., et al., *Disruption of IRS-2 causes type 2 diabetes in mice*. Nature, 1998. **391**: p. 900-904.

34. Morris, E.J. and H.M. Geller, *Induction of neuronal apoptosis by camptothecin, an inhibitor of DNA topoisomerase-I: evidence for cell cycle-independent toxicity*. The Journal of Cell Biology, 1996. **134**: p. 757-770.

35. Shafi, R., et al., *The O-GlcNAc transferase gene resides on the X chromosome and is essential for embryonic stem cell viability and mouse ontogeny*. Proc. Natl. Acad. Sci. U.S.A., 2000. **97**(11): p. 5735-9.

36. Natesan, S., et al., *Transcriptional squelchiing re-examined*. Nature, 1997. **390**: p. 349-350.

37. Bowe, D.B., et al., *O-GlcNAc Integrates the Proteasome and Transcriptome To Regulate Nuclear Hormone Receptors*. Molecular and Cellular Biology, 2006. **26**(22): p. 8539-8550.

38. Yang, X., F. Zhang, and J. Kudlow, *Recruitment of O-GlcNAc transferase to Promoters by corepressor mSin3a: Coupling protein O-GlcNAcylation to Transcriptional repression*. Cell, 2002. **110**: p. 69-80.

39. Williame-Morawek, S., et al., *IGF-1 protects cortical neurons against ceramide-induced apoptosis via activation of the PI-3K/Akt and ERK pathways; is this protection independent of CREB and Bcl-2?* Molecular Brain Research, 2005. **142**: p. 97-106.

40. Zheng, W.-H. and R. Quirion, *Insulin-like growth factor-1 (IGF-1) induces the activation/phosphorylation of Akt kinase and cAMP response element binding protein (CREB) by activating different signaling pathways in PC12 cells*. BMC neuroscience, 2006. **7**(51): p. 1-10.

41. Kreppel, L.K., M.A. Blomberg, and G.W. Hart, *Dynamic Glycosylation of Nuclear and Cytosolic Proteins*. The Journal of Biological Chemistry, 1997. **272**(14): p. 9308-9315.