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New Concepts

Metabolism-Independent Sugar Effects on Gene Transcription: The Role of 3-O-Methylglucose†

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ABSTRACT: Glucose effects on cellular functions such as gene expression require, in general, glucose metabolism at least to glucose-6-phosphate (G-6-P). However, the example of thioredoxin-interacting protein (TXNIP), a glucose-regulated gene involved in the cellular redox state and pancreatic beta cell apoptosis, demonstrates that this rule may not always apply. We found that aside form glucose, the nonmetabolizable sugars 2-deoxyglucose, which is still converted to G-6-P as well as 3-*O*-methylglucose (3-MG), which cannot be phosphorylated by glucokinase, stimulate TXNIP expression. In contrast, incubation of INS-1 beta cells with equimolar amounts (25 mM) of l-glucose or mannitol had no effect on TXNIP expression as measured by real-time RT-PCR, eliminating the possibility of an osmotic effect. Also, glucose uptake into the cell is critical because phloretin, an inhibitor of glucose transporter 2, blunted the glucose effects. Moreover, the 3-MG effect was not restricted to a cell line and was observed in 293 cells and primary human islets. Incubation of INS-1 cells with 30mM mannoheptulose, an inhibitor of glucose metabolism, blunted all glucose-induced gene expression but left the 3-MG effects unaltered. Using transient transfection studies and deletion constructs of the human TXNIP promoter, we found that the effects of glucose and 3-MG were dependent on the same region of the TXNIP promoter containing an E-box repeat carbohydrate response element (ChoRE). Thus, these findings provide the first evidence for regulation of gene expression by 3-MG, which is independent of glucose metabolism and suggest that glucose and 3-MG regulate transcription by two distinct pathways converging at a common ChoRE.

Glucose affects multiple cellular processes including gene expression. Almost all of these effects and especially those on transcription are dependent on glucose metabolism (*1*−*5*). Glucose enters the cell with the help of glucose transporters and, in a first critical step, is phosphorylated by glucokinase to G-6-P[1]. G-6-P can then continue in the glycolytic pathway or enter the pentose phosphate shunt. Surprisingly, we recently found that the expression of thioredoxin-interacting protein (TXNIP) is not only strongly stimulated by glucose (*6*, *7*) but also by the nonmetabolizable sugars 2-deoxyglucose (2-DG), which is still converted to G-6-P, as well as by 3-MG, which cannot be phosphorylated by glucokinase (*6*). TXNIP, also called vitamin D3-upregulated gene 1 (VDUP1), is a ubiquitously expressed gene that binds and inhibits thioredoxin and thereby regulates the cellular redox state (*8*). TXNIP is also involved in the inhibition of proliferation and stimulation of apoptosis, and thus, its regulation has far-reaching cell biological consequences (*9*). In addition, TXNIP represents the first exa-

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[1] Abbreviations: G-6-P, glucose-6-phosphate; TXNIP, thioredoxin-interacting protein; 3-MG, 3-*O*-methylglucose; 2-DG, 2-deoxyglucose; ChoRE, carbohydrate response element; ACC, acetyl-CoA carboxylase; GLUT2, glucose transporter 2; FBS, fetal bovine serum.

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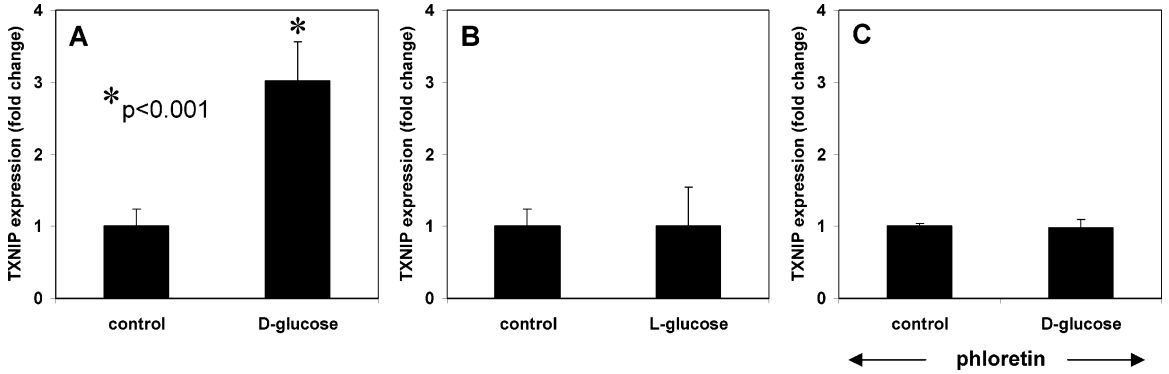

FIGURE 1: Glucose-induced TXNIP expression is not osmotic and requires glucose transport. INS-1 cells were incubated in 25 mM d-glucose, 25 mM l-glucose, or 2.5 mM glucose (control) for 6 h. To block glucose transport, cells were pretreated for 1 h with 300 μM phloretin. The bars represent mean fold change in the TXNIP expression of two independent experiments analyzed in triplicate ± SEM as measured by quantitative real-time RT-PCR. The *p* value was calculated by two-sided Student's *t*-test.

mple of a gene regulated by 3-MG. The present study was, therefore, aimed at investigating whether metabolism is necessary for the observed sugar effects on TXNIP expression and what the mechanisms are that mediate this unexpected 3-MG effect.

EXPERIMENTAL PROCEDURES

Reagents. Mannoheptulose was obtained from Glycoteam (Hamburg, Germany). l-glucose and d-glucose were from Fisher (Acros Organics) (Fair Lawn, NJ). Phloretin, Rotenone, Mannitol, 2-DG, and 3-MG were obtained from Sigma (St. Louis, MO).

Cell Culture. INS-1 cells were maintained in RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, 1 mM sodium pyruvate, 2 mM l-glutamine, 10 mM HEPES, and 0.05 mM 2-mercaptoethanol. To check for an osmotic effect, cells were exposed to 25 mM of l-glucose or mannitol for 6 h. Glucose transport was inhibited by pretreatment with the glucose transporter 2 (GLUT2) inhibitor, phloretin (300 μM) for 1 h (*10*). Glucose metabolism was blocked by 30 mM mannoheptulose, a glucokinase inhibitor (*3*−*5*). INS-1 cells were grown overnight in serum-free RPMI 1640 containing 0.1% BSA and 2.5 mM glucose and then incubated in the presence or absence of mannoheptulose starting 1 h prior to the 6 h treatment with 0 mM or 25 mM glucose, 2-DG, or 3-MG.

Human islets were a generous gift from Dr. David Harlan, NIDDK, National Institutes of Health. Islets were incubated for 6 h at no glucose or 25 mM 3-MG, after an overnight culture in RPMI 1640 containing 0.1% BSA and 2.5 mM glucose.

293 cells were cultured in DMEM (Invitrogen) with 10% FBS and 1% penicillin-streptomycin before the 6 h incubation in serum-free DMEM with no glucose, 25mM glucose, 2-DG, or 3-MG.

HIT-T15 cells were grown in RPMI 1640 containing 11.1 mM glucose supplemented with 10% FBS and 1% penicillin-streptomycin.

Quantitative Real-Time RT-PCR. RNA was extracted using TRIzol (Invitrogen) according to the manufacturer's instructions and converted to cDNA with the First Strand cDNA synthesis kit (Roche). Quantitative real-time RT-PCR was performed on a Prism 7000 Sequence Detection System (Applied Biosystems). Rat TXNIP was quantified with the forward primer 5′-CGAGTCAAAGCCGTCAGGAT-3′, the reverse primer 5′-TTCATAGCGCAAGTAGTCCAAGGT-3′, and the probe 5′-CTCAGCAGTGCAAACA-3′. Human TXNIP was amplified with the forward primer 5′-ACTCGT-GTCAAAGCCGTTAGG-3′ and the reverse primer 5′-TCCCTGCATCCAAAGCACTT-3′, as described previously (*6*). Acetyl-CoA carboxylase (ACC) expression was quantified using the forward primer 5′-GATGGCAGCAGTTA-CACCACAT-3′ and the reverse primer 5′-GCCAATTGT-GATTCGGTATCTGT-3′. All samples were analyzed in triplicate and corrected for the 18S ribosomal subunit (Applied Biosystems) run as an internal standard.

Western Blotting. Whole cell extracts of INS-1 and 293 cells were prepared and analyzed for TXNIP protein expression as described previously using the JY2 anti-TXNIP antibody (MBL International Co.) and beta-actin (Abcam) as a loading control (*11*).

Transfection Studies. The different deletion constructs of the human TXNIP promoter were generated and cloned upstream of the firefly luciferase reporter gene into the pGL3 enhancer vector (*6*), and HIT-T15 cells were transfected with these constructs and the pRL-TK (Promega) control plasmid using Fugene 6 (Roche, Indianapolis, IN), as described previously (*6*). Three hours after transfection, media were changed to RPMI media containing 10% FBS and 1% penicillin-streptomycin, and the cells were incubated at 0 mM or 25 mM glucose or at 25 mM 3-MG. Cells were harvested 24 h after transfection, and firefly as well as renilla luciferase activity was determined using the Dual Luciferase Assay Kit (Promega).

Statistical Analysis. P values were calculated by Student's test or by one-way ANOVA for data sets of more than two groups.

RESULTS

To determine whether the previously observed sugar-induced increase in TXNIP expression was due to an osmotic effect, we used rat insulinoma INS-1 beta cells again and exposed them to equimolar amounts of l-glucose (Figure 1B) or mannitol (data not shown). In contrast to d-glucose, which caused a > 3-fold increase (Figure 1A), neither of these treatments affected TXNIP expression, indicating that it is not an osmotic stimulus that confers increased TXNIP expression in response to sugar. In parallel to the effects on

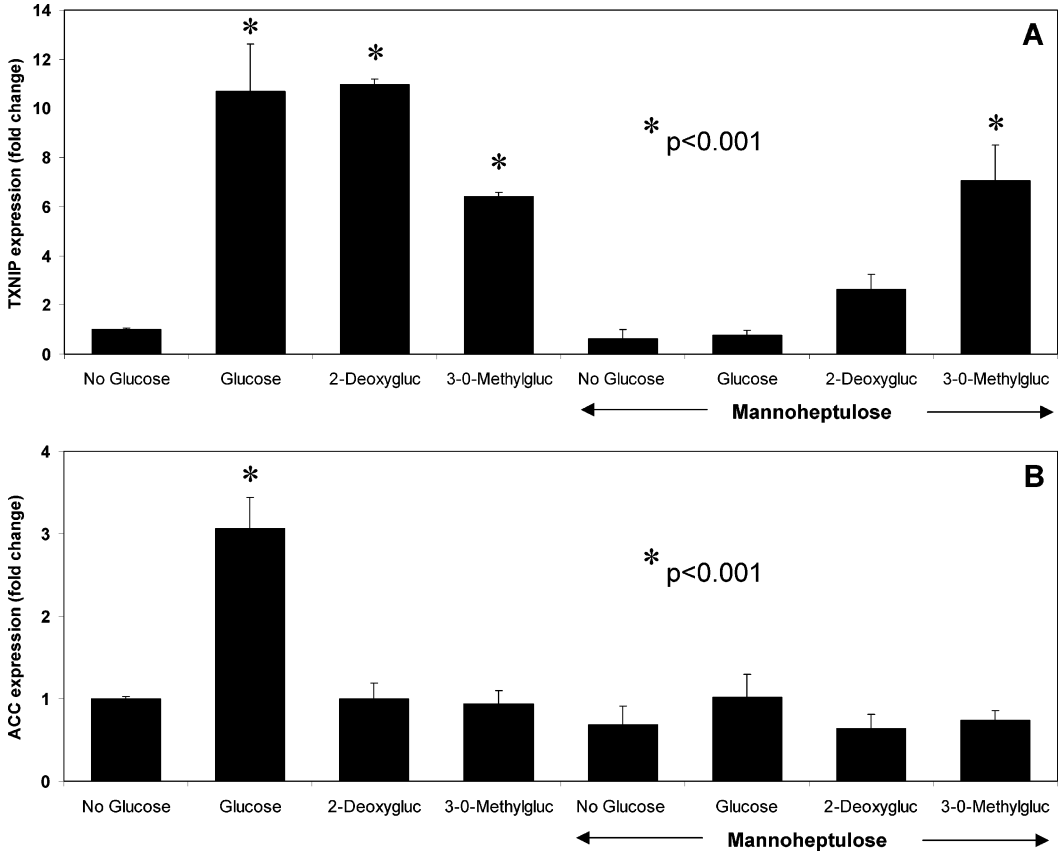

FIGURE 2: Mannoheptulose blocks glucose but not 3-*O*-methylglucose effects on TXNIP expression. INS-1 cells were incubated in the presence or absence of the glucose metabolism inhibitor, mannoheptulose (30 mM), and exposed to no glucose, 25 mM glucose, 25 mM 2-DG, or 25 mM 3-MG for 6 h. The bars represent fold change ± SEM in the expression of (A) TXNIP and (B) ACC as assessed by real-time RT-PCR. The means of at least three independent experiments analyzed in triplicate are shown. *P* values were calculated by one-way ANOVA.

TXNIP mRNA, we also observed a 4.6-fold increase in TXNIP protein levels in response to 25 mM D-glucose as measured by Western blotting and corrected for beta-actin (data not shown). To further assess the importance of glucose transport in this phenomenon, we blocked GLUT2 by phloretin, which abolished all glucose effects (Figure 1C). This suggests that glucose transport into the cell is essential for glucose-induced TXNIP expression. In contrast, rotenone, an inhibitor of mitochondrial electron transport, did not affect glucose-induced TXNIP expression (data not shown), making a contribution from mitochondrial metabolism unlikely.

To assess whether the observed TXNIP induction in response to glucose and nonmetabolizable sugars also occurs in other cell lines, we treated human embryonic 293 kidney cells and again observed a 3.1 ± 0.1-fold increase in TXNIP expression in response to 25 mM glucose and a 26.7 ± 2.9-fold increase in response to 25 mM 3-MG ($p < 0.001$). Western blotting also revealed an ~3-fold increase in TXNIP protein levels in 293 cells incubated again for just 6 h with these sugars (data not shown). A demonstration of these effects in this nonbeta cell line suggests that insulin secretion is not involved, which is consistent with our previous findings indicating that insulin does not stimulate TXNIP expression (*6*).

Incubation of isolated intact human islets with 25 mM 3-MG also led to a dramatic 47.4 ± 1.7-fold increase in TXNIP expression compared to that of the no glucose control ($p < 0.001$), indicating that the 3-MG effect is not a cell line phenomenon and that it also occurs in primary human islets.

On the basis of these results, our previous findings (*6*), and the fact that 3-MG is not metabolized and, therefore, commonly used to determine the role of metabolism in glucose-mediated effects (*3*), one would conclude that glucose metabolism is not required for the observed glucose-induced TXNIP expression. However, because this would represent a very unusual phenomenon, we wanted to obtain direct evidence for it. To this end, we blocked glucose metabolism with mannoheptulose and treated INS-1 cells again with glucose, 2-DG, and 3-MG (Figure 2). Interestingly, glucose- and 2-DG-induced TXNIP expression was blocked by mannoheptulose, suggesting that glucose metabolism was important for these effects. (Although the 2-DG effect was not completely abolished, TXNIP expression was not significantly different from the no glucose control; $p = 0.7$.) In contrast, mannoheptulose did not inhibit the 3-MG-mediated induction of TXNIP expression (Figure 2A) as expected, given the fact that 3-MG is not metabolized. Measuring ACC expression as an example of a glucose-regulated gene requiring glucose metabolism (*3*) in the same samples showed, as expected, no induction in response to 3-MG and inhibition of glucose-induced ACC expression by mannoheptulose (Figure 2B). To avoid any confounding effects from glucose present in the regular culture media, cells were kept at 0 mM glucose for these experiments, and together, this makes any contribution from a potentially unaccounted presence of glucose very unlikely.

To investigate the mechanism by which 3-MG led to the unexpected induction of TXNIP expression, we compared

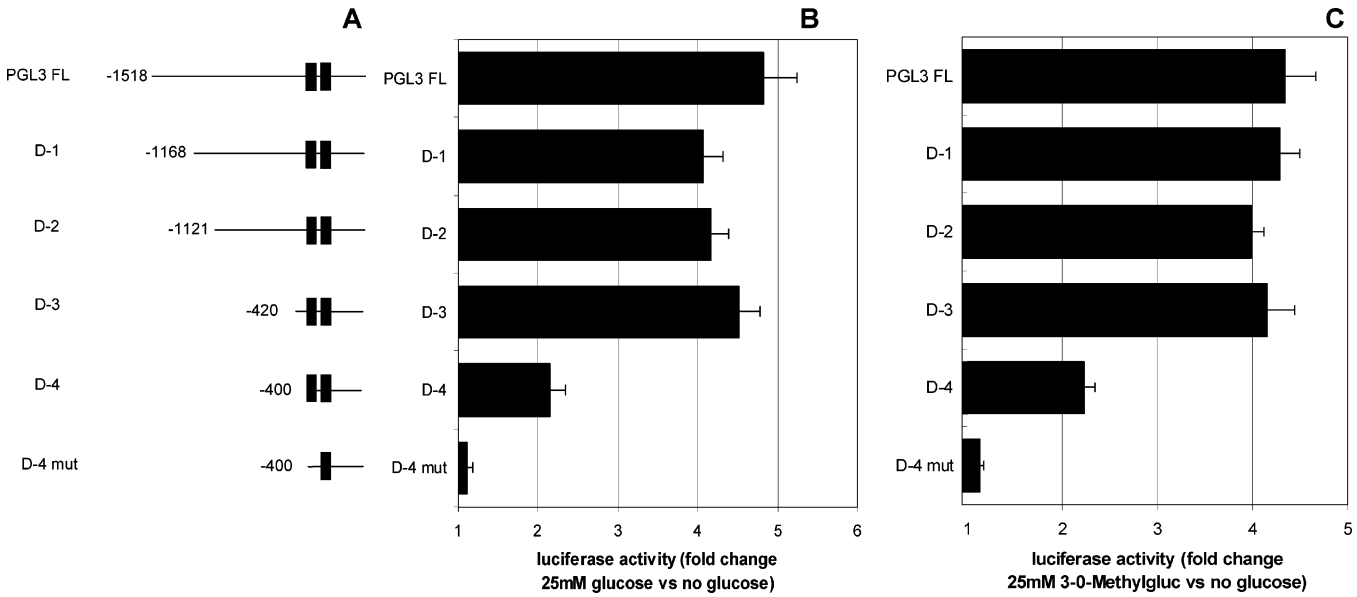

FIGURE 3: Glucose and 3-*O*-methylglucose induce TXNIP transcription through a ChoRE in the TXNIP promoter. (A) Schematic representation of the human TXNIP promoter deletion constructs (PGL3 FL−D-4 mut). The numbers refer to base pairs upstream of the start codon. The black boxes represent the two E-boxes of the carbohydrate response element (ChoRE). (B) Glucose effects on TXNIP promoter activity. HIT-T15 cells were transfected with the TXNIP promoter reporter constructs, and the bars represent mean fold change ± SEM in luciferase activity at 25 mM glucose compared to that of no glucose. (C) 3-MG effects on TXNIP promoter activity. Cells were transfected as described in B but incubated in 25 mM 3-MG or no glucose. The bars represent mean fold change ± SEM in luciferase activity in response to 3-MG of three independent experiments performed in duplicate.

the effects of 3-MG and glucose on the transcriptional activity of the human TXNIP promoter (Figure 3). These transfection studies revealed that the proximal 420 bp region of the promoter was sufficient to maintain an ~4.5-fold increase in transcriptional activity in response to both glucose (Figure 3B) and 3-MG (Figure 3C). Further deletion of 20 bp caused a decline in sugar-induced transactivation, and mutation of the first E-box of the ChoRE described previously (*6*) resulted in total ablation of the induction. These results demonstrate that glucose and 3-MG stimulate TXNIP expression at the transcriptional level and that the effects of both sugars are mediated by the same region of the TXNIP promoter containing the (CACGAGnnnnnCACGAG) E-box repeat ChoRE. Taken together, this suggests that glucose and 3-MG induce TXNIP expression by two distinct pathways (metabolism dependent and independent, respectively) and that both pathways converge at the level of the cis-acting element of the TXNIP promoter (Figure 4).

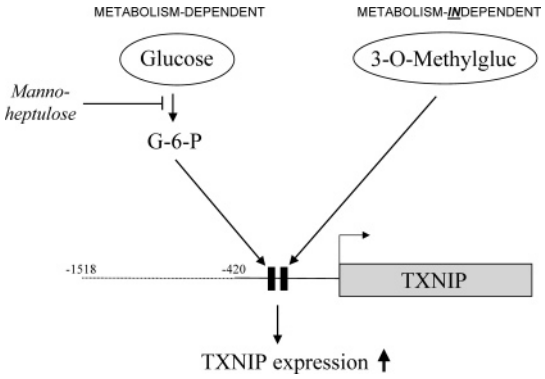

FIGURE 4: Schematic representation of the glucose metabolism-dependent and -independent pathways stimulating TXNIP expression. Although both glucose and 3-MG induce the expression of TXNIP at the transcriptional level, the effect of glucose is dependent on glucose metabolism as demonstrated by the fact that mannoheptulose completely blunted the effect. In contrast, the 3-MG effect is not dependent on metabolism. This suggests that the pathways involved in these effects are different. However, they seem to converge at the level of the cis-acting element because both sugars require the proximal 420 bp region in the TXNIP promoter containing the two E-boxes of the ChoRE.

DISCUSSION

The present study provides the first evidence for sugar-induced gene transcription independent of metabolism. Our data demonstrate that the nonmetabolizable 3-MG induces TXNIP expression in rodent and human cell lines as well as in primary human islets.

The greater magnitude of the 3-MG effect in human islets and 293 cells as compared to INS-1 cells could be due to a number of different reasons. INS-1 cells are derived from a rat insulinoma and are, therefore, tumor cells, which could explain their lower responsiveness. In addition, compared to other cell types, basal TXNIP expression level is ~3-fold higher in INS-1 cells as estimated by real-time RT-PCR, and this may decrease the dynamic range for a potential TXNIP increase in these cells. Furthermore, INS-1 cells are of rat origin, whereas 293 cells and the isolated primary islets used were of human origin, suggesting that there may be species differences. Considering our promoter findings and the highly conserved E-box repeat, it is tempting to speculate that species differences in the intervening 5 bp and/or upstream 20 bp, especially between rats and humans (*6*), might contribute to the observed difference in 3-MG effect size. This could provide helpful clues for future studies aimed at identifying the exact nucleotide sequence, specifically affecting the 3-MG effects.

The fact that the 3-MG effects were not blocked by mannoheptulose further confirmed that metabolism was not involved. At the same time, experiments using l-glucose and mannitol ruled out an osmotic effect. Intriguingly, we found

that the underlying mechanism for the observed 3-MG effects is based on transcriptional activation mediated by a unique ChoRE in the TXNIP promoter, the same as that used by glucose (*6*). This suggests that this 3-MG effect is highly specific, and indeed, it was restricted to TXNIP and not observed with ACC, another glucose-regulated gene (*3*). However, TXNIP may just be the first candidate of a class of genes regulated by nonmetabolizable sugars, and it is likely that the expression of other genes is regulated in a similar fashion. One interesting feature that distinguishes TXNIP from other glucose-regulated genes is that its ChoRE is nonpalindromic (*6*), and it is, therefore, tempting to speculate that this might allow for the observed 3-MG effect.

Our 3-MG findings are in alignment with the idea that elevated intracellular glucose levels *per se* can cause a number of cellular changes, as recently discussed in the context of diabetic complications (*12*). In addition, the hypothesis of metabolism-independent effects has recently been raised on the basis of findings that mannoheptulose did not block glucose-induced stabilization of glucokinase (*13*).

Consistent with the widely established notion that most glucose effects require glucose metabolism (*14*), but unexpected given the 3-MG effects, we found that glucose-induced TXNIP expression was dependent on glucose metabolism and that the effect was completely blunted by mannoheptulose. These findings emphasize the importance of directly blocking glucose metabolism with mannoheptulose, before assuming that a process does not require metabolism. The presence of a 3-MG effect may not always provide an accurate reflection of the glucose pathways involved, especially because 3-MG can act as a potent regulator of ChoRE-mediated transcription. This also might cause interference when using 3-MG to assess different cellular processes such as glucose transport (*14*).

In summary, our findings suggest that glucose and 3-MG regulate TXNIP transcription by two distinct pathways converging at a common ChoRE and thereby introduce the novel concept of a gene being regulated by glucose metabolism-dependent as well as -independent mechanisms.

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REFERENCES

1. Vaulont, S., Vasseur-Cognet, M., and Kahn, A. (2000) Glucose regulation of gene transcription, *J. Biol. Chem. 275*, 31555−31558.
2. Roche, E., Assimacopoulos-Jeannet, F., Witters, L. A., Perruchoud, B., Yaney, G., Corkey, B., Asfari, M., and Prentki, M. (1997) Induction by glucose of genes coding for glycolytic enzymes in a pancreatic beta-cell line (INS-1), *J. Biol. Chem. 272*, 3091−3098.
3. Brun, T., Roche, E., Kim, K. H., and Prentki, M. (1993) Glucose regulates acetyl-CoA carboxylase gene expression in a pancreatic beta-cell line (INS-1), *J. Biol. Chem. 268*, 18905−18911.
4. Roduit, R., Morin, J., Masse, F., Segall, L., Roche, E., Newgard, C. B., Assimacopoulos-Jeannet, F., and Prentki, M. (2000) Glucose down-regulates the expression of the peroxisome proliferator-activated receptor-alpha gene in the pancreatic beta-cell, *J. Biol. Chem. 275*, 35799−35806.
5. Marie, S., Diaz-Guerra, M. J., Miquerol, L., Kahn, A., and Iynedjian, P. B. (1993) The pyruvate kinase gene as a model for studies of glucose-dependent regulation of gene expression in the endocrine pancreatic beta-cell type, *J. Biol. Chem. 268*, 23881−23890.
6. Minn, A. H., Hafele, C., and Shalev, A. (2005) Thioredoxin-interacting protein is stimulated by glucose through a carbohydrate response element and induces beta-cell apoptosis, *Endocrinology 146*, 2397−2405.
7. Shalev, A., Pise-Masison, C. A., Radonovich, M., Hoffmann, S. C., Hirshberg, B., Brady, J. N., and Harlan, D. M. (2002) Oligonucleotide microarray analysis of intact human pancreatic islets: identification of glucose-responsive genes and a highly regulated TGFbeta signaling pathway, *Endocrinology 143*, 3695−3698.
8. Nishiyama, A., Masutani, H., Nakamura, H., Nishinaka, Y., and Yodoi, J. (2001) Redox regulation by thioredoxin and thioredoxin-binding proteins, *IUBMB Life 52*, 29−33.
9. Schulze, P. C., De Keulenaer, G. W., Yoshioka, J., Kassik, K. A., and Lee, R. T. (2002) Vitamin D3-upregulated protein-1 (VDUP-1) regulates redox-dependent vascular smooth muscle cell proliferation through interaction with thioredoxin, *Circ. Res. 91*, 689−695.
10. Hosokawa, M., and Thorens, B. (2002) Glucose release from GLUT2-null hepatocytes: characterization of a major and a minor pathway, *Am. J. Physiol. Endocrinol. Metab. 282*, E794−E801.
11. Chen, J., Couto, F. M., Minn, A. H., and Shalev, A. (2006) Exenatide inhibits beta-cell apoptosis by decreasing thioredoxin-interacting protein, *Biochem. Biophys. Res. Commun. 346*, 1067−1074.
12. Brownlee, M. (2005) The pathology of diabetic complications: a unifying mechanism, *Diabetes 54*, 1615−1625.
13. Zelent, D., Najafi, H., Odili, S., Buettger, C., Weik-Collins, H., Li, C., Doliba, N., Grimsby, J., and Matschinsky, F. M. (2005) Glucokinase and glucose homeostasis: proven concepts and new ideas, *Biochem. Soc. Trans. 33*, 306−310.
14. Levine, K. B., Robichaud, T. K., Hamill, S., Sultzman, L. A., and Carruthers, A. (2005) Properties of the human erythrocyte glucose transport protein are determined by cellular context, *Biochemistry 44*, 5606−5616.

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