TCF-4 Mediates Cell Type-specific Regulation of Proglucagon Gene Expression by β-Catenin and Glycogen Synthase Kinase-3β

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The proglucagon gene (glu) encodes glucagon, expressed in pancreatic islets, and the insulinotropic hormone GLP-1, expressed in the intestines. These two hormones exert critical and opposite effects on blood glucose homeostasis. An intriguing question that remains to be answered is whether and how glu gene expression is regulated in a cell type-specific manner. We reported previously that the glu gene promoter in gut endocrine cell lines was stimulated by β-catenin, the major effector of the Wnt signaling pathway, whereas glu mRNA expression and GLP-1 synthesis were activated via inhibition of glycogen synthase kinase-3β, the major negative modulator of the Wnt pathway (Ni, Z., Anini, Y., Fang, X., Mills, G. B., Brubaker, P. L., & Jin, T. (2003) J. Biol. Chem. 278, 1380–1387). We now show that β-catenin and the glycogen synthase kinase-3β inhibitor lithium do not activate glu mRNA or glu promoter expression in pancreatic cell lines. In the intestinal GLUTag cell line, but not in the pancreatic InR1-G9 cell line, the glu promoter G2 enhancer-element was activated by lithium treatment via a TCF-binding motif. TCF-4 is abundantly expressed in the gut but not in pancreatic islets. Furthermore, both TCF-4 and β-catenin bind to the glu gene promoter, as detected by chromatin immunoprecipitation. Finally, stable introduction of dominant-negative TCF-4 into the GLUTag cell line repressed basal glu mRNA expression and abolished the effect of lithium on glu mRNA expression and GLP-1 synthesis. We have therefore identified a unique mechanism that regulates glu expression in gut endocrine cells only. Tissue-specific expression of TCF factors thus may play a role in the diversity of the Wnt pathway.

Extensive research in cancer biology and embryogenesis has led to identification of the Wnt signaling pathway (1–7). A major effector of this pathway is the bipartite transcription factor cat/TCF,1 formed by the heterodimerization of β-catenin (β-cat) with one of the four TCF/LEF factors (TCF1, LEF-1, TCF-3, and TCF-4) (6). Activation of this pathway occurs via various mechanisms, including inactivation of the serine/threonine kinase GSK-3β, by Wnt signals (6), lithium (8), or other inhibitory factors (9), leading to the accumulation of free β-cat. Free β-cat accumulation may also occur as the consequence of mutations in several molecules in the Wnt signaling network including β-cat itself. More than 60 potential target genes have now been identified for cat/TCF, and the biological role of the Wnt pathway extends far beyond the scope of oncology and embryology.

The proglucagon gene (glu) is expressed in pancreatic islet α cells, intestinal endocrine L cells, and selected neural cells in the brain (10). Although the identical glu mRNA and pro-hormone are generated in these three tissues, post-translational processing leads to the cell/tissue-specific biosynthesis of three major peptide hormones, glucagon, glucagon-like peptide-1 (GLP-1), and GLP-2. These three peptide hormones exert diametrically opposed or overlapping biological functions (11–13). Glucagon, synthesized in the pancreatic α cells, is a counter-regulatory hormone to insulin. It is important in the maintenance of normoglycemia, particularly during fasting. In contrast, GLP-1, produced in the gut and brain, lowers blood sugar levels through stimulation of insulin secretion and biosynthesis, inhibition of glucagon release and gastric emptying, enhancement of peripheral insulin sensitivity, and importantly, induction of satiety (10, 14–16). Although the major function of GLP-2 is considered a growth factor for the small intestinal epithelium (17), recent studies indicate that it may also possess overlapping function with GLP-1 in the regulation of food intake (18). Exploring the molecular mechanisms that underlie cell type-specific expression of the glu gene may therefore lead to the development of novel approaches for the treatment and prevention of diabetes and obesity.

We present in this study the identification of a molecular mechanism that specifically regulates glu gene expression in the gut endocrine L cells and suggest that the glu gene in gut endocrine L cells, but not in the pancreatic islet α cells, is a downstream target gene of cat/TCF.

EXPERIMENTAL PROCEDURES
Plasmids—All proglucagon (GLU)/Luciferase (LUC) reporter gene plasmids have been described previously (19–21), or are illustrated in Fig. 2b. G2-TK-LUC constructs were generated by inserting one copy of the corresponding element into the parental TK-LUC fusion gene (20). The S33Y mutant β-cat expression plasmid and TCF-4 dominant-negative retrovirus expression system (pPGS-dnTCF-4 and the empty vector) were gifts from Dr. Eric Fearon (22). The pTOPFLASH LUC fusion gene plasmid and the wild-type TCF-4 expression plasmid were gifts from Dr. Eric Fearon (22).

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1 The abbreviations used are: TCF, T cell factor; LEF, lymphoid enhancer-binding factor; β-cat, β-catenin; glu, proglucagon gene; GLP, glucagon-like peptide; RT, reverse transcription; ChIP, chromatin immunoprecipitation; GSK-3β, glycogen synthase kinase-3β; DN, dominant-negative; LUC, luciferase.

This paper is available online at http://www.jbc.org
from Dr. Bert Vogelstein (23, 24). The GSK-3β expression plasmid was provided by Dr. James Woodgett (University of Toronto).

Cell Culture, Cell Transfection, and LUC Reporter Gene Analysis—Intestinal proglucagon producing GLUTag and STC-1 cell lines and pancreatic proglucagon producing InR1-G9 and α-TC-1 cell lines were grown in Dulbecco’s modified Eagle’s medium supplemented with appropriate serum (Invitrogen) (19). Methods for examining the effects of lithium on GLU-LUC reporter gene expression, glu mRNA expression, and GLP-1 synthesis have been described previously (25). Plasmid DNA pPGR-dnTCF-4 or the empty vector were transfected into GLUTag cells and GLP-1 synthesis have been described previously (25). Plasmid DNA ing and by Southern blotting with the corresponding DNA probe. Corresponded to the regions of interest was obtained by DNA sequencing—TCF binding site. Confirmation that the fragments generated in PCR part of the Exon 1 sequence of the mouse ATTCGTATCCCAGATCAG-3 expression in the gut GLUTag and STC-1 endocrine cell lines, as well as in primary fetal rat intestinal cell cultures (25). The

RESULTS

Lithium Treatment Does Not Activate glu mRNA Expression in Pancreatic Islet Cell Lines—We have demonstrated previously that the GSK-3β inhibitor lithium stimulates glu mRNA expression in the gut GLUTag and STC-1 endocrine cell lines, as well as in primary fetal rat intestinal cell cultures (25). The

![Fig. 1](image_url)
genes: G2L-TK-LUC, G2S-TK-LUC, and G2M-TK-LUC. G2L (long) represents the full-length wild-type G2 enhancer element (29), whereas G2S (short) is 8 bp shorter than G2L, although the TCF binding site remains unchanged. G2M (mutant) is identical with G2S, except for the presence of a disabling mutation in the TCF binding site.

When the three GLU-LUC fusion genes were transfected into the gut GLUTag cell line, lithium treatment was found to significantly activate the expression of both G2L-TK-LUC and G2S-TK-LUC but not the expression of G2M-TK-LUC (Fig. 3b). In contrast, lithium treatment generated no detectable effect on G2S-TK-LUC activity when transfected into the pancreatic InR1-G9 cell line. Furthermore, the expression of both G2L-TK-LUC and G2M-TK-LUC was repressed by lithium treatment when transfected into the InR1-G9 cell line (Fig. 3b).

The reporter gene G2S-TK-LUC was then used to examine the effect of co-transfection with S33Y-β-cat, a constitutively active mutant that cannot be destroyed by GSK-3β (22, 25). In this study, the parental TK-LUC construct was included to examine whether G2S itself could serve as an enhancer element for the TK promoter. As shown in Fig. 3c, when transfected into the GLUTag cell line, the LUC reporter gene activity of G2S-TK-LUC was 2-fold higher than that of the parental TK-LUC. When transfected into the InR1-G9 cell line, however, the LUC reporter gene activity of G2S-TK-LUC was similar to that of the parental TK-LUC fusion gene. These observations suggest that G2S serves as an enhancer element for gut proglucagon-producing cells, but not for the pancreatic proglucagon-producing cells. Co-transfection with the constitutively active S33Y-β-cat also generated a significant activation of G2S-TK-LUC in the GLUTag cell line but not in the InR1-G9 cell line (Fig. 3c).

If the major effect of lithium treatment on glu promoter expression occurs via the inhibition of GSK-3β, one would expect that overexpression of GSK-3β might attenuate the effect of lithium. Consistent with this hypothesis, we found that co-transfection of GSK-3β with G2S-TK-LUC or pTOPFLASH (in a 5:1 ratio) completely abolished the activation mediated by lithium treatment (Fig. 3d). Taken together, our observations suggest that TCF-E within the G2 enhancer element mediates the activation of glu gene expression by lithium treatment and by S33Y-β-cat co-transfection, specifically in the gut proglucagon-producing cells.

**TCF-4 Binds to glu Promoter and Is Expressed in Gut but Not in Pancreatic Endocrine Cells**—Among the four known TCF factors that bind β-cat, TCF-4 is the major mediator of Wnt signals in colon cancer cells (24). Furthermore, in the absence of free β-cat, TCF factors may serve as negative modulators of gene expression because they lack the activation domains but are still able to occupy TCF binding sites on cat/TCF target promotors. Indeed, we found that wild-type TCF-4 cDNA repressed the ~302 bp GLU-LUC fusion gene when co-transfected into the GLUTag cell line (data not shown).

ChIP assay was then used to examine whether TCF-4 and β-cat in GLUTag cell line bind to the glu gene promoter. Fig. 4a is a schematic presentation of the two pairs of PCR primers used in the ChIP assay, whereas Fig. 4b shows a representative ChIP assay result. The experimental primers amplify the DNA sequence from -43 bp to -340 bp of the mouse glu gene, a fragment that contains the G2 enhancer element. The control primers amplify a 205-bp DNA fragment within exon I of the mouse glu gene. This region contains no TCF binding site. Two different anti-TCF-4 antibodies and an anti-β-cat antibody were able to precipitate chromatin DNA that contains the G2 element, whereas the anti-c-Myc antibody could not. The same anti-c-Myc antibody has been previously used in examining the in vivo binding of c-Myc to CCL6 gene promoter (28). It is interesting that the anti-LEF-1 antibody was also able to precipitate chromatin containing the G2 enhancer element.

We then examined the expression of TCF-4 in proglucagon-producing cell lines. As shown in Fig. 5a, TCF-4 mRNA expression was detected by RT-PCR in the GLUTag cell line (lane 1) as well as in the human SW480 colon cancer cell line (as positive control; lane 3) but was not detected in the pancreatic InR1-G9 cell line (lane 2). Fig. 5b further shows the detection of TCF-4 protein in two gut proglucagon-producing cell lines, GLUTag (lane 1) and STC-1 (lane 2), and in the human colon cancer cell line SW480 (lane 5), by Western blotting. TCF-4 protein expression in the two pancreatic α-cell lines was barely detectable (lanes 3 and 4). Finally, immunostaining was performed in both proglucagon-producing cell lines as well as in murine paraffin-embedded tissue sections. Immunofluorescence staining showed that TCF-4 is abundantly expressed in the GLUTag and STC-1 cell lines but was barely detectable in the InR1-G9 and α-TC-1 cell lines (Fig. 6a). Consistent with these findings, TCF-4 immunoreactivity was also identified in the nucleus of most intestinal epithelial cells but was absent in pancreatic islets (Fig. 6b). Co-expression of TCF-4 and GLP-1 was then demonstrated in the GLUTag cell line by immunofluorescence staining (Fig. 6c, right). The pancreatic InR1-G9 cells also expressed small amount of immunoreactive GLP-1, because of abnormal post-translational processing of the proglucagon in this cell line, but did not express TCF-4 (Fig. 6c, left).

**Dominant-Negative TCF-4 Represses Basal glu mRNA Expression and Disrupts the Activation Mediated by Lithium in GLUTag Cells**—To further elucidate the role of TCF-4 in regulating gut glu mRNA expression and GLP-1 synthesis, we stably transfected GLUTag cells with a dominant-negative (DN) TCF-4 construct that lacks the N-terminal β-cat binding site (22) (Fig. 7a). This approach has been successfully used by Kolligs et al. (22) to study S33Y-β-cat-mediated neoplastic transformation. Northern blotting analysis showed that basal glu mRNA expression was substantially reduced in GLUTag-TCF-4(DN) cells compared with either wild-type GLUTag cells or GLUTag cells stably transfected with the empty retroviral vector (GLUTag-TCF-4(V); Fig. 7b). Furthermore, glu mRNA expression in the GLUTag-TCF-4(DN) cells showed no response to lithium treatment. We then compared the effect of lithium treatment on expression of the LUC reporter gene constructs pTOPFLASH and G2S-TK-LUC in GLUTag-TCF-4(DN) and GLUTag-TCF-4(V) cells. When pTOPFLASH was examined, GLUTag-TCF-4(DN) cells showed no response to lithium treatment, whereas GLUTag-TCF-4(V) cells demonstrated ~10-fold activation by lithium (Fig. 7c, left). Likewise, when G2S-TK-LUC was examined, the activation by lithium...
The effect of lithium treatment on GLP-1 synthesis in GLUTag-TCF-4(DN) cells was less than 1.5-fold, compared with greater than 3-fold activation in GLUTag-TCF-4(V) cells (Fig. 7d, right). The increases in GLP-1 synthesis in response to lithium treatment were also observed in GLUTag-TCF-4(V) cells. However, GLUTag-TCF-4(DN) cells completely lost the response to lithium (Fig. 8). These observations strongly indicate the involvement of TCF-4 in activation of both glu mRNA expression and GLP-1 synthesis in gut endocrine cells by inhibiting GSK-3β with lithium.

Finally, we asked whether the lack of the response in GLUTag-TCF-4(DN) cells was due to an overall decrease in GLUTag-TCF-4 expression. We found that lithium treatment increased GLUTag-TCF-4(V) expression by 5-fold in GLUTag cells (Fig. 9d, right). However, GLUTag-TCF-4(DN) cells completely lost the response to lithium (Fig. 9d). Our results suggest that TCF-4 is necessary for the activation of glu gene expression and GLP-1 synthesis by inhibiting GSK-3β with lithium.

**FIG. 3.** TCF-E mediates cell type-specific activation of the glu promoter by lithium and S33Y β-cat. a, overall organization of the rat glu gene promoter, showing TCF/LEF binding sites (WWGTTTC) and a cAMP-response-element (CRE). G2L, G2S, and G2M represent long, short, and mutant G2 elements, respectively. The TCF/LEF binding site within the G2 enhancer element is conserved between mouse, rat, and human. b, 3 μg of G2L-TK-LUC, G2S-TK-LUC, or G2M-TK-LUC was transfected into the intestinal GLUTag or pancreatic InR1-G9 cell lines. Lithium chloride (final concentration, 10 mM) was added 24 h before harvesting for LUC reporter gene analysis. Relative LUC activities were calculated as -fold induction relative to a corresponding control (mean ± S.D., n = 3, *, p < 0.05; **, p < 0.01; ***, p < 0.001). c, the indicated LUC reporter gene plasmids (0.5 μg) were co-transfected into the GLUTag or InR1-G9 cell lines with 2.5 μg of pCDNA3 or S33Y-β-cat. Cells were harvested 24 h after the transfection for LUC reporter gene analysis. d, G2S-TK-LUC or pTOPFLASH (0.5 μg) were co-transfected into the GLUTag cell line with 2.5 μg pCDNA3 or GSK-3β (in the pCDNA3 expression plasmid). Cells were treated with lithium chloride (final concentration, 10 mM) 24 h before harvesting for LUC reporter gene analysis. Relative LUC activities were calculated as -fold induction relative to a corresponding control (mean ± S.D., n = 3, *, p < 0.05; **, p < 0.01; ***, p < 0.001).
Because the glu gene encodes three major peptide hormones that play critical roles in blood glucose homeostasis and in controlling satiety (11–13), extensive research has been conducted to elucidate the molecular mechanisms that underlie expression of this gene (29–41). These studies have identified nearly a dozen transcription factors and signaling molecules. To date, however, none of these factors/molecules has been found to regulate glu gene expression in intestinal cells only or in pancreatic cells only. For example, the homeodomain protein Cdx-2 was found to activate glu gene promoter in both pancreatic and intestinal proglucagon-producing cell lines (20, 41). Although overexpression of Cdx-2 stimulates endogenous glu mRNA expression in the pancreatic InR1-G9 cells (41) but not in the intestinal GLUTag cells (40), the involvement of Cdx-2 in regulating basal glu mRNA expression in both pancreatic and intestinal cells cannot be excluded. Likewise, the paired homeodomain protein Pax-6 (39, 40) and the protein kinase A signaling pathway (21, 26, 30, 35) have been found to regulate glu gene expression in both pancreatic and intestinal proglucagon-producing cell lines, as well as in primary pancreatic islet and intestinal cell cultures.

We demonstrate here that lithium treatment activates glu gene promoter and glu mRNA expression in the gut endocrine cell lines but not in pancreatic islet proglucagon-producing cell lines. We reported previously that lithium also activates glu mRNA expression and GLP-1 synthesis in primary fetal rat intestinal cell cultures (25). Because this cell type-specific activation is dependent on the TCF binding motif within the G2 enhancer element and the tissue-specific expression of TCF-4, we suggest that the glu gene in gut endocrine cells, but not in pancreatic islet cells, is a down-stream target of cat/TCF-4.

Future studies will be required to determine which Wnt molecule(s) regulates glu gene expression in the gut endocrine cells and whether glu expression in the brain is also regulated by lithium and the Wnt pathway. Peripheral administration of lithium was found to reduce food/water intake, decrease salt ingestion after sodium depletion, and induce robust conditioned taste aversion in rats (42–49). These effects can be mimicked by central (intracerebroventricular) administration of GLP-1 (14). More importantly, the effects provoked by lithium can be blocked by pre-treating the experimental animals with GLP-1 receptor antagonists (48, 49). It is therefore reasonable to hypothesize that lithium may mediate satiety by up-regulating the production of brain GLP-1.

The majority of studies to identify transcription factors and signaling molecules that regulate glu gene expression in gut and pancreas have been conducted using cultivated cell lines. A challenging question that remains is whether the cultivated cell lines used in these studies are good surrogates of the primary glu producing cells. Our previous studies have demonstrated that the GLUTag cell line responds appropriately to many factors/molecules that regulate glu gene expression and GLP-1 synthesis and secretion in primary gut endocrine cells, including cAMP/protein kinase A, glucose-dependent insulinotropic peptide, and bethanechol (26, 40, 50, 51). Likewise, the α-TC-1 and InR1-G9 cell lines have been routinely used as α cell models to study glu gene expression and glucagon synthesis and secretion (19, 20, 30, 52–56). In these cell lines, for example, glucagon secretion is repressed by retinol and retinoic acid (52) and activated by phorbol esters (54), cytosolic calcium oscillations are inhibited by high glucose, and glu mRNA expression and glucagon synthesis are inhibited by insulin (55). Thus, these cell lines are believed to be good models of the primary gut and pancreatic proglucagon-expressing cells.

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TCF-4 Mediates Gut Proglucagon Gene Expression

DISCUSSION

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TCF-4 is expressed in the normal epithelia of the small and
large intestines, whereas its expression in pancreatic islets has not been reported (57). We recently isolated primary pancreatic islet cells by laser capture microscope from adult mice. RT-PCR approach shows that both the mouse primary pancreatic islet cells and the two cultivated islet cell lines express glu mRNA as well as mRNA for several glu gene transactivators, including Cdx-2 and Pdx-6 (data not shown) but not TCF-4. This observation, in combination with our immunostaining results (shown in Fig. 6b), suggests that TCF-4 is indeed not expressed in pancreatic islets. Consistent with a previous report (58), we found that TCF-4 immunoreactivity was weaker in jejunum and stronger in ileum and colon (Fig. 6b). Although TCF-4 deletion mutants (TCF72/−/− mice) have been previously generated, these mice die shortly after birth (58). A single histopathological abnormality has been described in the intestines of these mice: they lack a proliferative compartment in the prospective crypt regions between the villi, despite a normal transition of intestinal endoderm into the epithelium at e14.5 (58). It will be interesting to examine the potential involvement of TCF-4 and other TCF factors in the genesis of the gut GLP-1-producing endocrine cells using the TCF-4 deficient mice.

To our knowledge, there is no previous report on the binding of a transcription factor to the glu gene promoter by ChIP

**FIG. 6.** Detection of TCF-4 expression in cultivated cell lines and in paraffin-embedded pancreatic and intestine tissue samples. a, detection of TCF-4 immunoactivity in cultivated cell lines using a rabbit polyclonal anti-TCF-4 (diluted 1:100) with a fluorescein isothiocyanate-conjugated anti-rabbit IgG (green). Cell nuclei were stained with DAPI (blue). b, detection of TCF-4 expression in paraffin-embedded mouse tissue sections. Tissue sections were incubated with a polyclonal anti-TCF-4 antibody diluted 1:200, followed by incubation with a horseradish peroxidase-conjugated secondary antibody and visualization using diaminobenzidine as the substrate. 1, jejunum, control; 2, ileum, control; 3, colon, control; 4, jejunum, TCF-4; 5, ileum, TCF-4; 6, colon, TCF-4; 7, islet, control; 8, islet, TCF-4. c, co-immunostaining of TCF-4 and GLP-1. GLUTag and InR1-G9 cells were incubated with the mouse monoclonal anti-TCF-4 antibody (6H5–3) and the rabbit polyclonal anti-GLP-1 antibody. The primary antibodies were then detected by fluorescein isothiocyanate-conjugated anti-rabbit IgG antibody (green) and TRIkie-conjugated anti-mouse IgG antibody (red). Cell nuclei were stained with 4,6-diamidino-2-phenylindole (blue). Cells were viewed with a LSM510 confocal microscope.
assay. We made an unsuccessful attempt to examine the binding of TCF-4 and βH9252-cat to the G2 enhancer element by electrophoretic mobility shift assay. However, although ChIP assay reveals the binding events that occur in intact cells rather than in a test tube, a limitation of this technique is that it cannot precisely define the binding site on the target gene promoters.

In our case, for example, we cannot eliminate the possible involvement of TCF-D, which is only 263 bp upstream of TCF-E (Fig. 3a). Nevertheless, results from our LUC reporter gene analyses shown in Figs. 3, b–d, and 7c strongly support the involvement of TCF-E in regulating lithium and S33Y/βH9252-catenin-mediated cell type-specific activation. The ChIP assay also suggested the binding of LEF-1 to the mouse glu gene promoter (Fig. 4b). We have conducted preliminary RT-PCR analysis that indicates expression of LEF-1 and TCF-3 in the GLUTag cell line (data not shown). The expression and the function of TCF factors, other than TCF-4, in both intestinal and pancreatic proglucagon-producing cells therefore need to be further investigated.

The Wnt pathway represents one of the most complicated regulatory networks in metazoa, characterized by the existence of multiple native ligands and receptors, as well as more than a dozen other related components, including four different TCF proteins. Although different TCF factors have been implicated in many different biological events, tissue-specific expression of a given TCF factor in governing gene expression in cell-specific manner has not been well documented. To further appreciate the role of TCF-4 in regulating GLP-1 synthesis, it

FIG. 7. Stable expression of DN TCF-4 abolishes lithium-induced activation of glu mRNA and promoter expression. a, a schematic representation of TCF-4 showing the β-cat binding site that was deleted in the TCF-4(DN) construct (22). The high mobility group box (HMG_box) represents the DNA binding domain of TCF-4. b, the indicated GLUTag cells were treated with control media (–) or media with 10 mM lithium chloride for 24 h. Total RNA was extracted, and glu mRNA expression was examined by Northern blotting. The same membranes were then stripped and rehybridized with a probe against tubulin. Approximately 10 μg of RNA was loaded for each sample. Glu, proglucagon; Tub, tubulin. c, effects of lithium on pTOP-FLASH (left) and G2S-TK-LUC (right) when transfected into the GLUTag-TCF-4(V) and the GLUTag-TCF-4(DN) cells. 3 μg of the indicated reporter gene plasmid was transfected into the indicated cell line. Lithium chloride (final concentration, 10 mM) was added 24 h before harvesting for LUC reporter gene analysis. Relative LUC activities were calculated as fold induction relative to a corresponding control (mean ± S.D., n ≥ 3. *, p < 0.05; **, p < 0.01; ***, p < 0.001).

FIG. 8. Stable expression of DN TCF-4 abolishes lithium-induced activation of GLP-1 synthesis. Activation of GLP-1 synthesis by lithium treatment (final concentration, 10 mM) was examined in GLUTag-TCF-4(DN) cells, compared with both the wild-type GLUTag and GLUTag-TCF-4(V) cells. Relative GLP-1 synthesis was calculated as a percentage relative to cells that received no treatment (mean ± S.D., n ≥ 3. *, p < 0.05; **, p < 0.01; ***, p < 0.001).
will be necessary to examine the expression and function of TCF-4 in gut glu-expressing cells in response to various nutritional and other physiological factors.

In addition to acting as the key negative modulator of Wnt signals, GSK-3β is also an important negative regulator of insulin action, and its activity is elevated in fat and muscle tissues from murine models of type 2 diabetes as well as in human patients (59, 60). The development of new generations of GSK-3β inhibitors may therefore provide novel approaches for diabetes treatment (61, 62). We found recently that another GSK-3β inhibitor, 4-benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione, also activated glu mRNA expression in the GLUTag cell line. Understanding the role of GSK-3β inactivation in stimulating the production of the insulinotropic hormone GLP-1 may further the potential for the therapeutic use of GSK-3 inhibitors in human diseases.

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