Glucose Metabolism to Glucosamine Is Necessary for Glucose Stimulation of Transforming Growth Factor-α Gene Transcription*

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Transforming growth factor-α (TGF-α) gene transcription can be increased when arterial smooth muscle cells are exposed to supraphysiological concentrations of glucose, and this effect of glucose can be mimicked by glucosamine. To determine whether the metabolism of glucose to glucosamine is required for this glucose effect, the rate-limiting step in glucose metabolism to glucosamine through the enzyme glutamine:fructose-6-phosphate amidotransferase (GFAT) was blocked using pharmacological and antisense strategies. We found that blockage of GFAT activity or expression significantly blunted the glucose-induced increase of TGF-α expression. Blockage of GFAT also resulted in a decreased RL2 signal on intracellular proteins as detected by Western blotting and indirect immunofluorescence. The RL2 monoclonal antibody recognizes an epitope on proteins that contain N-acetylglicosamine and thus is a measure of protein glycosylation. Conversely, treatment of the cells with glucose and glucosamine resulted in an increase in the RL2 epitope on intracellular proteins. These results indicate that the metabolism of glucose to glucosamine is necessary for the transcriptional stimulation of TGF-α expression in vascular smooth muscle cells by glucose. Furthermore, the level of glycosylation of some intracellular proteins can be modulated in response to physiological changes in the extracellular glucose concentration and the net activity of GFAT.

Many of the long term complications of diabetes mellitus relate to vascular pathology, and in type I diabetes, there is now strong epidemiologic evidence linking hyperglycemia to vascular disease (1, 2). The vascular smooth muscle cell plays a pivotal role in the development of vascular pathology; proliferation of these cells is an early event in the pathogenesis of atherosclerosis. Growth factors have been hypothesized to play an important role in the control of this process (3). Previous studies have localized TGF-α and basic fibroblast growth factor to these same vascular smooth muscle cells (4–6). These proteins are known to induce mitogenic stimulation of endothelial and smooth muscle cells as well as having acute vasoactive effects (7). With regard to diabetes, it has recently been demonstrated that the expression of these growth factors can be increased at the transcriptional level by placing vascular smooth muscle cells in supraphysiological concentrations of glucose (8). A known metabolite of glucose and product of the hexosamine pathway, glucosamine, mimics this effect of glucose in stimulating growth factor gene expression, but glucosamine stimulates a greater response than glucose and at lower concentrations (8). It is believed that the rate-limiting enzyme in glucosamine synthesis is glutamine:fructose-6-phosphate amidotransferase (GFAT). When the yeast GFAT cDNA was transiently overexpressed in vascular smooth muscle cells, the glucose induction of TGF-α expression was augmented 2-fold (9). The addition of glucosamine to the cell culture medium (10) and overexpression of GFAT (9) both result in an increase in the intracellular concentration of uridine diphosphate-N-acetylglucosamine (UDP-GlcNAC), a substrate for protein glycosylation. Thus, the experiments testing the effects of glucosamine or GFAT overexpression on TGF-α gene transcription both indicate that an increase in the intracellular UDP-GlcNAC pool can result in increased TGF-α gene transcription. However, such experiments do not prove that glucose must be metabolized through GFAT to glucosamine to exert its effect on growth factor gene transcription.

To determine if GFAT is necessary for the glucose induction of TGF-α gene transcription, an antisense approach was taken in which the expression of GFAT was blocked and the effect of this blockade on glucose signaling of TGF-α gene transcription was measured. This approach presented some difficulties because GFAT is an allosterically regulated enzyme that is negatively feedback regulated by its product, UDP-GlcNAC (11, 12). Thus, any incomplete blockade of GFAT expression would allow the remaining enzyme to compensate with an increase in specific activity, thereby frustrating our goal of blocking glucosamine synthesis. Thus, our experiments had two essential requirements. The first was to achieve extremely high levels of antisense RNA to prevent a compensatory response by GFAT. The second was to prove that glucosamine synthesis in these cells indeed had been blocked. The first requirement was satisfied with the use of a strongly inducible promoter, the HIV-LTR. The activity of this promoter is high in the basal state but can be induced a further 1000-fold by the HIV transactivator, Tat (13). The second requirement took advantage of the fact that perturbations in the size of the UDP-GlcNAC pool are reflected in the level of glycosylation of intracellular proteins (14). Glycosylation of proteins is achieved by the transfer of the GlcNAc moiety from UDP-GlcNAC to proteins by cellular transferases (15–17). This attachment to proteins is both through N- and O-linkage. Recent work has shown that the GlcNAC content of glycoproteins can be measured with the monoclonal antibody, RL2 (14), which appears to recognize O-linked GlcNAC (18). This property of RL2 allowed us to obtain a measure of the GlcNAC content of proteins in individual cells by immunofluorescence microscopy thus providing an assessment of the...
downstream consequences of changes in glucosamine synthesis resulting from blockade of GFAT activity or expression. Also, the use of RL2 allowed us to determine the effects of physiologically relevant concentrations of glucose on glycoprotein metabolism. From these experiments, we can now conclude that glucosamine synthesis from glucose is necessary for the induction of TGF α gene transcription by glucose and that changes in the ambient concentration of glucose through physiologically relevant concentrations does result in changes in the glycosylation of intracellular and nuclear proteins.

MATERIALS AND METHODS

Cell Culture—Rat aortic smooth muscle cells (RASM) were prepared from the aortas of 250–300-g male Sprague-Dawley rats (19). Culture media was from Life Technologies, Inc., and supplies were purchased from Sigma. The cells were cultured routinely in Dulbecco's modified Eagle's medium (DMEM) + 10% fetal calf serum (FCS) (5 mg glucose) and in the presence of 100 μM penicillin G and 50 μg/mL gentamicin. Cells were grown at 37 °C in a humidified incubator with 5% CO 2 . The cells were passed every 7 days and used for up to eight passages.

Plasmid Constructs and Electroporation—The plasmid containing the 5'-flanking sequence of the TGF α gene cloned upstream of the firefly luciferase gene (pTGF α-LUC) has already been described (20). The pSV-TAT vector has been described elsewhere (13). The HIV/ Antisense GFAT vector was generated by cutting HIV-LUC (13) with HindIII and -untranslated region was ligated with the HIV-Antisense vector. All plasmids were supercoiled and twice purified over CsCl gradients prior to transfections. Plasmids were used in the following amounts: 18 μg of pTGF α-LUC, 4 μg of pSV-TAT, and 20 μg of either pHIV/Antisense or pHIV/Control. Briefly, 6 × 10 5 cells were trypsinized, washed with phosphate-buffered saline, and resuspended in DMEM + 10% FCS. After adding the plasmids, cells were electroporated at 400 μF microfarads in a Bio-Rad Gene Pulser (Bio-Rad). Cells were plated in 6-well plates (Fisher) with 4.5 × 10 5 cells per well in media described in each figure. For glucose and glucosamine dose-response studies, cells were plated at 3.75 × 10 6 and 2.5 × 10 6 cells per well, respectively, using 12-well plates.

Assay of Luciferase Activity—Luciferase activity was measured in detergent extracts of cells in the presence of ATP and luciferin as described previously (20) using a Monolight 1000 luminometer (Analytical Luminescence Laboratory, San Diego, CA). Light output was integrated over a 10-s period and displayed as relative light units. Background activity was found to be less than 1% of all readings.

Northern Blot Analysis—Briefly, 5 × 10 5 cells were electroporated with the appropriate DNA constructs and plated on 10-cm plates. At 18 h post-transfection, cells were harvested, and RNA was isolated by the acid guanidine thiocyanate/phenol/chloroform method of extraction (22). The RNA was quantitated and 20 μg of total RNA from each condition was electrophoresed through 1% agarose, 6% formaldehyde, transferred, and UV-cross-linked onto GeneScreen nylon membranes (DuPont NEN). The membrane was probed overnight at 42 °C with cDNA which was labeled on both strands to a specific activity of greater than 3 × 10 8 cpm/μg using the large fragment of DNA polymerase I (23). The GFAT probe was a 1.0-kilobase fragment of the mouse GFAT cDNA (20), and a 1.6-kilobase fragment of β-actin was used as a control (24). Radiosamples, [ 32 P]dATP (1,000 Ci/mmole), and [ 32 P]dCTP (1,000 Ci/mmole) were purchased from DuPont NEN.

Western Blot Analysis—Using 10-cm plates at 70% confluency, cells were pretreated overnight with 40 μM 6-diazoo-5-oxonorleucine (DON) or vehicle control. The following morning, cells were stimulated with the appropriate sugar and lysed 48 h later. Briefly, each plate was scraped, and cells were pelleted at 500 × g at 4 °C. After several washes with cold saline, cells in detergent and plated in 10-cm plates. The supernatants were quantitated for protein concentration using the Bio-Rad D 6 Protein Assay (Bio-Rad) and confirmed by Coomassie Blue staining of SDS-polyacrylamide electrophoresis gels. Equal amounts of protein from each sample were separated on an 8% acrylamide–SDS-containing gel and transferred onto nitrocellulose membranes (Schleicher & Schuell). The signals were detected using the Enhanced Chemiluminescence System (Amersham Corp.) after incubation with the RL2 (18, 25) or Sp1 (26) antibodies.

Results and Discussion

Effect of GFAT Inhibition on Glucose- and Glucosamine-induced TGF α Transcription—Glucose stimulates TGF α transcription in vascular smooth muscle cells (8). Furthermore, this increase can be reproduced reliably in cells transfected with a construct containing the TGF α promoter upstream of the firefly luciferase reporter gene (8). Cells were treated with a known inhibitor of GFAT enzymatic activity, 6-diazo-5-oxonorleucine (DON), and its effect on TGF α transcription was monitored using this transient transfection reporter system. This glutamine analog covalently binds to and inactivates those enzymes that use glutamine in amidotransferase reactions such as GFAT (27). A pool of RASM cells, transfected with the TGF α-luciferase reporter gene, was divided into several treatment groups. The transfectedants were pretreated for 16 h with DON or vehicle alone. The following morning, glucose concentrations were left at 2.5 mM or increased to 30 mM, and cells were assayed for luciferase at the indicated times (Fig. 1A). In the absence of the inhibitor, glucose stimulated reporter function as previously reported (8, 9). However, treatment with 40 μM DON practically eliminated the effect of glucose on TGF α transcription. Glucosamine enters the hexosamine pathway downstream of GFAT. To determine whether glucose could overcome this effect of DON, the cells that had been transfected with the TGF α reporter were tested for the response to this sugar in the presence and absence of the GFAT inhibitor (Fig. 1B). In the absence of the inhibitor, glucose and glucosamine stimulated the TGF α reporter as usual (8), 3-fold with glucose and 7–12-fold with glucosamine. However, with 40 μM DON, the glucose-induced transcription was significantly reduced. Although the transcriptional response to glucosamine was slightly lower in the DON-treated cells than in the controls, it was still as vigorous a response as reported in the literature (8, 9), indicating that the transcriptional induction of the TGF α promoter remains essentially intact despite treatment of these cells with the GFAT inhibitor.

The HIV-LTR Drives High Level GFAT Antisense Expression—While the glutamine analog, DON, has previously been

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used as an inhibitor of GFAT activity, it potentially could have additional effects on other glutamine-utilizing pathways. To more specifically block GFAT activity, we used a GFAT-specific antisense mRNA strategy. In order to attain high antisense concentrations, we placed the GFAT cDNA containing the entire 5'-untranslated region and coding sequence in the antisense orientation under the control of the HIV-1-LTR (pHIV/Antisense). While the HIV-LTR is a very strong viral promoter, its activity can be transactivated severalthousandfold with the introduction of the HIV-1 Tat protein (13). We confirmed the ability of the HIV-1 Tat protein to transactivate the HIV-LTR in RASM cells. The tat cDNA under the control of the SV40 promoter (pSV-TAT) was transiently co-transfected along with the HIV promoter driving antisense GFAT mRNA, and Northern blot analysis was conducted on the transfected cells (Fig. 2). Although all lanes were loaded nearly equally with RNA, the cells transfected simultaneously with pSV-TAT, and pHIV/Antisense generated large amounts of antisense GFAT mRNA. There appears to be two transfection-dependent transcripts which are presumed to result from alternative splicing of the SV40 intron contained in the vector. Since the transient transfection efficiency in these cells is approximately 8% as determined by staining cells transfected with CMV-β-gal (data not shown), the content of antisense mRNA in the transfected cells is 10-fold higher than represented by this Northern blot.

Effect of Specific Antisense GFAT mRNA on Glucose-induced TGFα Transcription—Having demonstrated the high level production of antisense GFAT mRNA in RASM cells, we then determined the effect of this antisense on the ability of glucose to stimulate TGFα transcription. A time course study was performed to determine the optimal duration of antisense expression. Cells were transfected with the pTGFα-LUC reporter and pSV-TAT and pHIV/Antisense. The control cells were transfected with the same three plasmids except that the pHIV was devoid of the GFAT insert (pHIV/Control). Following transfection, the cells were allowed to recover for 15 h in standard medium and then the glucose concentrations were adjusted to 2.5 or 30 mM. Cells were assayed for luciferase at the indicated times following the adjustment of the glucose concentration (Fig. 3A). The ability of glucose to stimulate TGFα-luciferase activity in the presence of antisense GFAT mRNA was significantly reduced when compared with HIV/Control with the optimal effect observed at 48 h.

We then tested the effect of the antisense GFAT on the sensitivity of the TGFα promoter to glucose induction. In cells transfected with the control plasmid, the TGFα response to glucose was similar to previous observations (8, 9). TGFα reporter activity increased from a basal level of 20,000 units to a stimulated level of 80,000 units in response to glucose concentrations above 15 mM. The concentration of glucose that gave half-maximal stimulation (50,000 units) occurred at about 5 mM glucose. In the cells transfected with the antisense GFAT, the response to glucose was significantly blunted. While the basal reporter activity was 20,000 units, it increased to only 40,000 units. However, the half-maximal response of 30,000 units occurred at a glucose concentration of about 5 mM glucose, similar to the control cells (Fig. 3B). The TGFα transcriptional response to glucosamine stimulation on the same pool of trans-
fected cells was virtually unaffected by the expression of the antisense GFAT RNA (Fig. 3C). The sustained ability to respond to glucosamine in the cells containing the antisense GFAT RNA suggests that, by bypassing the GFAT step in the hexosamine pathway of glucose metabolism, the pathway leading to the TGF \( \alpha \) transcriptional response to glucose is intact distal to the GFAT step. Taken together, these results strongly support the notion that glucosamine synthesis from glucose is important for the TGF \( \alpha \) response to glucose.

**Analysis of Hexosamine Biosynthetic Products in the Presence and Absence of a GFAT Inhibitor**—Previous studies have shown that either overexpression of the GFAT enzyme or treatment of cells with glucosamine increase intracellular levels of UDP-GlcNAc (11, 12). Since UDP-GlcNAc serves as a substrate for protein glycosylation, we hypothesized that inhibition of GFAT by DON might decrease the level of glycosylation of proteins. It has been shown that the monoclonal antibody, RL2, can recognize O-linked GlcNAc moieties on proteins (14, 18, 25) on Western blots. Furthermore, when breast cancer cells were transferred from glucose-free to glucose or glucosamine containing media, and the intracellular proteins were analyzed by

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**FIG. 3.** Effect of antisense GFAT mRNA on the ability of glucose and glucosamine to stimulate TGF \( \alpha \) transcription. **A**, cells were transfected with pTGF \( \alpha \)-LUC, pSV-TAT, and either pHIV/Antisense or pHIV/Control. After overnight recovery, glucose concentrations were adjusted to 2.5 or 30 mM, and the transfected cells were assayed for luciferase activity at the indicated times after glucose stimulation. Results are the means \( \pm \) S.D. of two experiments, each assayed in triplicate on independent cultures and normalized to the values observed at 2.5 mM glucose at each point. *, \( p < 0.05; **, p < 0.0005; ****, p < 0.0025 (Student’s t test). **B**, cells were transfected with pTGF \( \alpha \)-LUC, pSV-TAT, and either pHIV/Antisense or pHIV/Control. After overnight recovery, the medium was aspirated and refreshed with DMEM \( + 10\% \) FCS (at the indicated glucose concentrations), and the cells were assayed for luciferase activity 48 h later. Results are the means \( \pm \) S.D. of a triplicate experiment. Differences between antisense (●) and control (○) transfected cells are significant at all data points (\( p < 0.025 \)) using Student’s t test. **C**, the same two pools of transfected cells (●, control; ●, antisense) were allowed to recover, refreshed with DMEM \( + 10\% \) FCS (2.5 mM glucose), and supplemented with glucosamine at the indicated concentrations. Cells were assayed for luciferase 72 h later. Results are the means \( \pm \) S.D. of a triplicate experiment.
RL2 Western blotting, the intensity of the RL2 signal was found to increase even when the cells were exposed to these sugars in the presence of the protein synthesis inhibitor, cycloheximide (14). This result suggested that changes in the intracellular UDP-GlcNAc pool could be reflected in the content of GlcNAc on intracellular proteins. To determine the effects of glucose and glucosamine on protein glycosylation in RASM cells in the presence and absence of DON, detergent-free extracts were prepared in order to enrich for cytosolic and nuclear proteins. Equal amounts of protein were loaded onto the gel, and the Western blot was probed with the RL2 antibody (Fig. 4). Multiple proteins were detected with RL2 and the intensity of the RL2 signal was augmented when the cells were cultured in high glucose. Exposure of cells to glucosamine resulted in a greater increase in the signal. However, when cells were cultured in the presence of 40 μM DON, and either 2.5 or 30 mM glucose, the RL2 signals were greatly reduced. However, when cultured in glucosamine, the RL2 signal on most of the protein bands did increase in the presence of 40 μM DON, although not to the same extent as seen in the cells treated with glucosamine alone. To control for protein loading, the blot was stripped and reprobed with an antibody directed at Sp1 (26), a nuclear protein known to harbor O-linked GlcNAc residues (28). While marked changes were noted in the RL2 signal under the various treatment conditions, the Sp1 signal remained relatively constant, suggesting that the content of GlcNAc in the proteins was changing rather than the abundance of the glycosylated proteins. This observation is in concert with a prior study that showed similar changes in the RL2 signal even in the absence of protein synthesis (14). These results indicate that changes in ambient glucose and glucosamine concentrations can alter intracellular protein glycosylation. Furthermore, it appears that GFAT is involved in the glucose stimulation of intracellular protein glycosylation as it can be inhibited with DON. Conversely, the glucosamine stimulation of protein glycosylation appears to occur largely independent of GFAT activity.

Cellular Localization and Quantification of the RL2 Signal by Indirect Immunofluorescence—In order to determine the cellular location of proteins that are detected by RL2 and undergo GlcNAc modification in response to glucose and glucosamine, we probed cells by indirect immunofluorescence with the RL2 antibody. Using this approach, we also determined the effect of treating the cells with DON, high glucose concentrations, or glucosamine (Fig. 5). In the absence of DON and in low glucose (2.5 mM), the RL2 signal was predominantly in the nucleus. However, some cytoplasmic staining was also evident. The intensity of the nuclear fluorescence was variable from cell to cell. Stimulation of these cells with 30 mM glucose generally augmented the nuclear signal. Exposure to glucosamine resulted in a dramatic increase in both the nuclear and cytoplasmic signals. In the presence of 40 μM DON, the fluorescence both in the low and high glucose-treated cells was significantly reduced. Similar to the observation with the Western blot, glucosamine treatment of the cells was able to restore much of the RL2 signal in the presence of the GFAT inhibitor. In order to quantitate the RL2 fluorescence, the slides were examined with a confocal microscope. This quantitation was performed

![Table 1](http://www.jbc.org/)

**Table 1** Relative fluorescent intensities of the RL2 signal of RASM cells shown in Fig. 5.

| Condition                  | 40 μM DON | Mean ± S.E. | N  |
|----------------------------|-----------|-------------|----|
| 2.5 mM Glc                 | −         | 30.85 ± 0.67 | 1439 |
| 30 mM Glc                  | −         | 46.59 ± 0.81 | 1300 |
| 2.5 mM Glc + 7 mM GlcN    | −         | 83.90 ± 1.06 | 1410 |
| 2.5 mM Glc                 | +         | 16.28 ± 0.43 | 1534 |
| 30 mM Glc                  | +         | 14.28 ± 0.40 | 1461 |
| 2.5 mM Glc + 7 mM GlcN    | +         | 58.61 ± 1.29 | 1310 |

* p < 0.001.
* p < 0.005.
* p < 0.01.
* p < 0.005.
because the serial photographic steps to produce Fig. 5 resulted in a loss of the contrast observed on the original microscope slides. For each of the six conditions shown, 15 individual nuclei were chosen at random by a uninformed technician from five different fields. The relative fluorescence values per given area of nucleus are listed in Table I. These data confirm that high extracellular glucose concentrations result in increased intracellular glycosylation as detected by RL2. The ability of glucose to increase the RL2 is entirely dependent upon GFAT, whereas the glucosamine effect is largely independent of this enzyme. The nuclear/cytoplasmic localization of the RL2 signal suggests that the proteins observed on the Western blot with this antibody are derived from these intracellular compartments. We speculate that the GlcNAc modifications detected by RL2 are most likely O-linked GlcNAc, a protein modification that is observed predominantly in the nucleus and cytoplasm (15–17).

RL2 Immunofluorescence of Antisense GFAT mRNA-transfected Cells—Having demonstrated that the activity of GFAT is critical for glucose-induced changes in the level of intracellular protein glycosylation, we could then test the effectiveness of the antisense GFAT at blocking the net activity of this enzyme. Since the antisense approach used transient transfection, only a limited number of cells were actually transfected (8%), necessitating an in situ assessment of GFAT activity. As shown in Fig. 5, blocking the activity of GFAT results in a detectably reduced RL2 signal in cells as determined by indirect immunofluorescence. Therefore, we used this approach to assess the downstream consequence of GFAT activity (RL2 signal) in cells that were cotransfected with pH1V/Antisense and pSV-TAT. Under these conditions of cotransfection, at least 95% of the cells expressing Tat will also express antisense GFAT mRNA (29). Thus, the transiently transfected cells could be identified by indirect immunofluorescence for the presence or absence of Tat protein. The RL2 signal in these Tat-positive cells, which expressed high levels of the antisense GFAT, was then compared with the RL2 signal in cells not expressing Tat. The Tat-negative cells thus served as a negative control for the antisense cells. The RL2 staining of cells grown in high glucose for 48 h following transfection is shown in Fig. 6A. Fields were selected to allow the examination of non-overlapping cells. A single cell in this typical field (arrowhead) displays a reduced RL2 immunofluorescence signal. While the RL2 signal varied from cell to cell (Fig. 5), we never observed a cell with as attenuated an RL2 signal as seen in this antisense experiment.

Fig. 6. Double immunofluorescence for RL2 and Tat in antisense GFAT mRNA-transfected cells. RASM cells were transfected with pH1V/Antisense and pSV-TAT. After overnight pretreatment in DMEM +10% FCS (2.5 mM glucose), the cells were stimulated with either 30 mM glucose (A and B) or 7 mM glucosamine (C and D). 48 h following the addition of the sugars, cells were fixed and analyzed by immunofluorescence as described. The cells stimulated with high glucose were examined for RL2 (A) and the same field of cells was examined for Tat expression (B). Similarly transfected cells that had been stimulated with glucosamine were similarly examined for RL2 (C) and Tat (D) immunofluorescence. Three independent experiments yielded 11 similar images for glucose-stimulated cells and nine images for glucosamine-treated cells. The calibration bar corresponds to 20 μm.
other fields, and these results were observed in an additional two independent experiments. Cells treated with glucosamine displayed no difference in the RL2 signal (Fig. 6C) whether the cell was positive or negative for Tat immunofluorescence (Fig. 6D). These results were also reproduced in an additional two experiments. When cells were cotransfected with pSV-TAT and HIV/Control instead of pHIV/Antisense, the RL2 staining was indistinguishable in Tat-positive and Tat-negative cells when cultured either in 30 mM glucose (Fig. 7) or 7 mM glucosamine (data not shown). Thus, the reduction in the RL2 signal did not result from Tat expression alone, but required co-expression of the antisense GFAT. These data further support the idea that glucose stimulation of intracellular protein glycosylation is GFAT-dependent while the effect of glucosamine on intracellular protein glycosylation is independent of GFAT activity.

In summary, the experiments described in this paper were designed to determine whether the metabolism of glucose to glucosamine is necessary for the stimulatory effect of glucose on TGF-α transcription in vascular smooth muscle cells. Two approaches were taken, one involving the pharmacological inhibition of GFAT activity and the other an antisense approach to block GFAT synthesis. These experiments indicated that the stimulation of TGF-α transcription by glucose largely does require its metabolism through GFAT to glucosamine. In addition, these experiments indicate that changes in the ambient glucose concentration from physiological to diabetic levels result in changes in nuclear and cytoplasmic protein glycosylation as detected with the RL2 antibody. Since prior studies (14) have indicated that these changes in the RL2 signal do not require new protein synthesis, these findings suggest that the stoichiometry of protein glycosylation is subject to regulation by glucose availability. The localization of the RL2 GlcNAc-containing epitope predominantly in the nucleus is compatible with the idea that RL2 recognizes O-GlcNAc. The O-GlcNAc modification is most abundant in nuclear proteins (15) and has been found not only in nuclear pore proteins but in many transcription factors (28, 30–32). That the RL2 signal can be modulated by physiologically relevant changes in the extracellular glucose concentration suggests a potential role for the O-GlcNAc modification in the regulation of gene transcription. However, thus far, there has been no functional change in a transcription factor associated with this modification. Nevertheless, the finding that the glucose effect on the transcription of the TGF-α gene is dependent on the metabolism of glucose to glucosamine is compatible with a role for protein glycosylation in transcription factor function.

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