Complete Inhibition of Glucose-induced Desensitization of the Glucose Transport System by Inhibitors of mRNA Synthesis

EVIDENCE FOR RAPID TURNOVER OF GLUTAMINE:FRUCTOSE-6-PHOSPHATE AMIDOTRANSFERASE*

Stephen Marshall$, Vincent Bacote, and Roger R. Traxinger§

From the Department of Biochemistry, University of Tennessee, Memphis, Tennessee 38163

Glutamine:fructose-6-phosphate amidotransferase (GFAT) plays a key role in desensitizing the insulin-responsive glucose transport system (GTS), and recent studies have revealed that loss of GFAT activity accompanies desensitization. To gain insights into the mechanisms underlying loss of enzyme activity, we have used primary cultured adipocytes and two well established inhibitors of mRNA synthesis to estimate GFAT turnover. Both actinomycin D and 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) caused a rapid and extensive loss in GFAT activity (>70% loss, t1/2 of 45 min) indicating that GFAT has a relatively short half-life. Since induction of insulin resistance requires GFAT, we next examined the ability of mRNA inhibitors to block glucose-induced desensitization. When adipocytes were cultured for 18 h with 20 mM glucose, amino acids, and 25 ng/ml insulin, maximal insulin responsiveness of the GTS was reduced by >70%. Both actinomycin D and DRB rapidly and completely prevented desensitization in a dose-dependent manner (ED50 of 16 nM and 15 μM, respectively). These findings are the predicted functional consequence of diminished GFAT activity. Evidence that actinomycin D acts selectively on GFAT without influencing other steps within the desensitization pathway was obtained using glucosamine, an agent that enters the hexosamine biosynthesis pathway at a point distal to the action of GFAT. Actinomycin D inhibited glucose-induced desensitization but failed to block glucosamine-induced desensitization. From these studies we conclude that 1) glucose-induced desensitization of the GTS can be completely prevented by actinomycin D and DRB, two potent and diverse inhibitors of mRNA synthesis; 2) the functional integrity of the desensitization pathway is maintained by a short-lived protein; and 3) the identity of this short-lived protein is most likely GFAT, the first and rate-limiting enzyme of the hexosamine biosynthesis pathway.

In the companion paper, we found that glutamine:fructose-6-phosphate amidotransferase (GFAT) is an insulin-regulated enzyme and that insulin-induced loss of GFAT activity is mediated by enhanced glucose uptake and the subsequent flux of glucose into the hexosamine biosynthesis pathway. Thus, a 4-h treatment with insulin alone or glucose alone failed to reduce GFAT activity, whereas combined treatment with insulin, glucose, and glutamine elicited a marked reduction in enzyme activity. Supporting the idea that formation of hexosamine products regulate GFAT activity was the finding that glucosamine, an agent known to directly enter the hexosamine pathway, effectively reduced GFAT activity in a dose-dependent manner. Since a close correlation was also seen between desensitization of the GTS and the loss of GFAT activity as a function of glucose, insulin, glutamine, and glucosamine concentrations (similar ED50 values), we concluded that both regulatory processes are tightly coupled to the formation of hexosamine products.

Although the mechanism(s) underlying loss of GFAT activity remains to be determined, several lines of evidence led us to believe that the progressive loss of enzyme activity is not due to allosteric regulation. First, we found that glucose-induced loss of GFAT activity occurs over several hours, rather than minutes as would be expected for allosteric regulation. Second, using [14C]-3-O-methylglucose and phloretin to measure the intracellular water space in adipocytes (1), we calculated that the cytosol was diluted more than 2,500-fold in the GFAT assay. Thus, it is unlikely allosteric effectors are involved in the glucose-induced loss of GFAT activity, since they would have been diluted beyond the point where allosteric regulation of GFAT could be observed. Last, the actual removal of potential allosteric regulators from the cytosol of desensitized cells by G-25 columns chromatography failed to restore GFAT activity to control values.

Through a process of elimination, we therefore postulated that the flux of glucose through the hexosamine biosynthesis pathway reduces GFAT activity by affecting mRNA levels and the steady-state levels of GFAT protein. Consistent with this idea is a recent study demonstrating that GFAT levels in yeast are under rapid transcriptional control; within 15 min after adding a mating pheromone, GFAT gene expression was increased 2-3-fold (2). If our hypothesis is correct and the coordinated regulation of GFAT activity by insulin, glucose, and glutamine is mediated through altered gene transcription, then it follows that the turnover of GFAT must be sufficiently rapid to account for the temporal loss of GFAT activity (t1/2.

* This work was funded in part by Research Grant DK38754 from the National Institutes of Health and Biomedical Research Support Grant RR05-5423. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

§ To whom correspondence should be addressed: University of Tennessee/Memphis, Dept. of Biochemistry, 800 Madison Ave., Memphis, TN 38163.

§§ Recipient of National Research Service Award DK08206 from the National Institutes of Health.

1 The abbreviations used are: GFAT, glutamine:fructose-6-phosphate amidotransferase; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HBSS, Hepes-buffered salt solution containing 25 mM Hepes and 1% bovine serum albumin, pH 7.4; MIR, maximum insulin responsiveness; GTS, glucose transport system; DRB, 5,6-dichlororibofuranosyl benzimidazole.
of 2 h. To test this idea, we blocked mRNA synthesis by means of two well-established mRNA synthesis inhibitors and then determined whether there was a progressive loss in glucose activity accompanied by the predicted attenuation of glucose-induced desensitization of the GTS. The obtained results are consistent with the idea that GFAT mRNA and protein have rapid turnover rates.

EXPERIMENTAL PROCEDURES

The materials and methods used were described in the preceding paper (3).

RESULTS

Effects of Actinomycin D on Glucose-induced Desensitization—Fig. 1 depicts an experiment in which adipocytes were suspended in Dulbecco's modified Eagle's medium (containing 20 mM glucose) and treated for 18 h (at 37°C) with the indicated concentrations of actinomycin D in either the absence or presence of 25 ng/ml insulin. At the end of the incubation period, adipocytes were thoroughly washed in insulin- and glucose-free buffer and both basal and maximally insulin-stimulated rates of glucose transport (MIR) were measured. In control cells (Fig. 1A), actinomycin D decreased basal rates of glucose uptake in a dose-dependent manner but had no effect on MIR. Thus, the extent of insulin stimulation was actually enhanced by actinomycin D treatment. In desensitized cells (Fig. 1B), low doses of actinomycin D completely prevented glucose-induced desensitization (ED50 of 16 nM) and blocked the decrease in basal glucose uptake (ED50 of 40 nM) that normally accompanies desensitization.

To determine whether the ability of actinomycin D to block desensitization could be reversed upon removal of this agent, we performed the experiment depicted in Fig. 2. Cells were pretreated with 200 nM actinomycin D for various times from 1 to 120 min, washed three times to remove actinomycin D, and then incubated with glucose, insulin, and amino acids to induce desensitization. After an 18-h desensitization period, adipocytes were again washed and MIR was assessed. From the results of this study two salient points emerged. First, on the basis of the time course of actinomycin D action, it appears that actinomycin D can rapidly block glucose-induced desensitization (t1/2 of 7 min). Second, the effects of actinomycin D are not reversible after treatment times of 30 min or longer (at least not within the 18-h time frame of the experiment). This finding is consistent with the mechanism of actinomycin D action in inhibiting mRNA synthesis. It is well established that actinomycin D can rapidly enter cells where it intercalates into the DNA in an irreversible manner (4, 5).

In Fig. 3 is a time course experiment examining the short-term effects of actinomycin D (200 nM) on glucose-induced desensitization. In this protocol, actinomycin D was added at the same time as the desensitizing agents. As can be seen, actinomycin D completely inhibited desensitization at very early times. For example, a 32% decrease in MIR was seen after 4 h of desensitization, and this loss of MIR was com-
completely prevented by actinomycin D treatment. On the basis of the established inhibitory action of actinomycin D on mRNA synthesis, we interpret these results to mean that glucose-induced desensitization depends on the continued synthesis of mRNA. Moreover, since actinomycin D was found to rapidly block desensitization, we believe that the desensitization pathway is maintained by a protein that is encoded by a mRNA with a very short half-life. It is noteworthy that actinomycin D was fully capable of blocking desensitization regardless of whether total amino acids or glutamine was used as the desensitization agent (Table I).

Effects of 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) on Glucose-induced Desensitization—To confirm that glucose-induced desensitization depends on a mechanism differing completely from that of actinomycin D (6-12). As shown in Fig. 4, when desensitized cells were treated for 18 h with various concentrations of DRB, desensitization was completely blocked at the higher doses (200 μM). In addition, the obtained EDso value of 15 μM agrees well with the results of published studies examining the dose-dependent ability of DRB to inhibit mRNA synthesis (10). As was true for actinomycin D, the onset of DRB action was very rapid (Fig. 5).

The inhibitory action of DRB on desensitization is completely reversible upon removal of this agent (Fig. 6). Thus, full desensitization was observed when cells were pretreated with 200 μM of DRB for various times from 5 to 120 min, washed in DRB-free buffer, and then treated with glucose, insulin, and amino acids for 4 h to induce desensitization. These results are in marked contrast to the irreversible action of actinomycin D (Fig. 2) but are in agreement with previous

### Table I

| Incubation conditions | 2-Deoxyglucose uptake (pmol/3 min) |
|-----------------------|-----------------------------------|
|                       | Total amino acids | Glutamine |
| Control               | 1205 ± 14          | 1147 ± 12 |
| Control + actinomycin D| 1203 ± 46          | 1119 ± 8  |
| Desensitized          | 694 ± 21           | 616 ± 19  |
| Desensitized + actinomycin D | 1200 ± 13        | 1205 ± 30 |

*p < 0.001, compared with control using the Student’s t test.

**Fig. 4.** Dose-dependent effects of 5,6-dichloro-1-β-D-ribofuranosyl benzimidazole on glucose-induced desensitization of the glucose transport system. Adipocytes were suspended in HBSS containing 20 mM glucose, 4 × amino acids, and 25 mg/ml insulin and incubated at 37 °C with the indicated concentrations of DRB. At the end of the 5-h incubation period, cells were washed and resuspended in glucose- and insulin-free HBSS and assayed to determine basal rates of 2-deoxyglucose uptake and MIR of the GTS. Control cells (A) were similarly treated under insulin-free conditions in absence or presence of 200 μM DRB. White bars represent basal rates of glucose uptake, and black bars reflect MIR. All data points and bars represent the mean ± S.E. of three experiments.

**Fig. 5.** Rapidity of 5,6-dichloro-1-β-D-ribofuranosyl benzimidazole in preventing glucose-induced desensitization (DSZ). Cells were pretreated with 200 μM DRB for various times from 0 to 60 min and then exposed to glucose (20 mM), amino acids (1 X), and insulin (25 ng/ml) to induce desensitization. After 4 h, all groups of cells were washed and MIR of the GTS was assayed. Control cells were similarly treated but were not exposed to DRB or insulin. Each bar represents the mean ± S.E. of four experiments.

**Fig. 6.** Reversibility of DRB action on glucose-induced desensitization (DSZ). Adipocytes were pretreated with 200 μM DRB for various times from 0 to 120 min, washed three times to remove DRB, and then exposed to glucose (20 mM), amino acids (1 X), and insulin (25 ng/ml) for 4 h to induce desensitization. At the end of 4 h, cells were again washed and basal (white bars) and MIR (black bars) of the GTS was measured. Control cells underwent similar treatment and washings, but after the washing procedure 200 μM DRB was re-added. Each bar represents the mean ± S.E. of three experiments.
studies demonstrating that the inhibitory effects of DRB on mRNA synthesis are completely reversible (10).

Effects of Actinomycin D and DRB on Glutamine:Fructose-6-P Amidotransferase Activity—To test the idea that actinomycin D blocks desensitization by reducing the enzymatic activity of GFAT, we measured GFAT activity at various times after exposing cells to 200 nM actinomycin D. As shown in Fig. 7, actinomycin D rapidly decreased GFAT activity by >70% with a t1/2 of about 45 min. In control cells, cytosolic enzyme levels remained constant throughout the 5-h incubation period. From these results, we conclude that actinomycin D prevents desensitization by rapidly reducing the synthesis of GFAT mRNA which in turn leads to a concomitant decrease in enzyme levels.

In a recent study, we concluded that de novo protein synthesis is not involved in the development of insulin resistance, since cycloheximide had no effect on glucose-induced desensitization of the GTS (13). To explain the paradox between this earlier finding and our more recent data indicating that GFAT has a rapid turnover rate, we performed an experiment in which adipocytes were incubated in the absence (controls) or presence of either 200 nM actinomycin D or 10 μg/ml cycloheximide (Fig. 7B). After 4 h, the cells were lysed and cytosolic GFAT activity was assessed. Because of the observed inability of cycloheximide to reduce GFAT activity, we concluded that cycloheximide failed to block glucose-induced desensitization, because GFAT levels were unaltered. If GFAT does indeed have a rapid turnover rate, then additional studies are clearly required to elucidate why inhibition of protein synthesis by cycloheximide fails to lower GFAT activity. One possibility that needs to be explored is the idea that turnover of GFAT is complex and regulated in part by de novo biosynthesis of labile proteins that modulate the half-life of GFAT or GFAT mRNA.

As depicted in Fig. 8, both actinomycin D and DRB significantly reduced GFAT activity after a 2-h treatment period; however, after washing cells free of these two agents, only those cells treated with 200 μM DRB were able to fully recover GFAT activity. Recovery of GFAT activity was not seen in actinomycin D-treated cells. The demonstrated ability of mRNA synthesis inhibitors to rapidly decrease GFAT activity (Fig. 7) and the equally rapid recovery of GFAT activity in DRB-treated cells (Fig. 8) add credence to the idea that the steady-state level of GFAT activity in adipocytes is maintained by a rapid equilibrium between GFAT biosynthesis and GFAT turnover.

Inability of Actinomycin D to Prevent Glucosamine-induced Desensitization of the GTS—Glucosamine has previously been shown to enter the hexosamine desensitization pathway at a point distal to GFAT action (14-17), and we recently found that glucosamine treatment of insulin-treated adipocytes could induce desensitization of the GTS (18). Therefore, we used glucosamine to determine whether mRNA synthesis inhibitors were acting on several steps within the hexosamine-desensitization pathway or whether they were acting selectively on GFAT activity. As can be clearly seen in Fig. 9, actinomycin D failed to block glucosamine-induced desensitization (A), whereas under identical conditions actinomycin D completely prevented glucose-induced desensitization (B). We infer from these results that actinomycin D selectively affects an early step in the hexosamine desensitization pathway (maintenance of GFAT levels) without altering the later events involved in the process of desensitization (all steps distal to the formation of glucosamine 6-phosphate).

Effects of Actinomycin D on the Formation of Hexosamine Products During Desensitization—When cells were treated

![Fig. 8. Recovery of glutamine:fructose-6-phosphate amidotransferase activity after treatment with 5,6-dichloro-1-beta-D-ribofuranosyl benzimidazole. Cells were treated for 2 h with either 200 nM actinomycin D (AD) or 200 μM DRB as described in Fig. 8. After washing adipocytes to remove the mRNA inhibitors, adipocytes were incubated for an additional 2 h (37 °C) after which time the enzymatic activity of cytosolic GFAT was determined. Each point represents the mean ± S.E. of four experiments.](image)

![Fig. 9. Inability of actinomycin D to prevent glucosamine-induced desensitization. A, cells were suspended in HBSS (containing 2 mM glucosamine and 1 × amino acids) in the absence (controls) or presence (desensitized) of 25 μg/ml insulin. Where indicated, 200 nM actinomycin D was added 30 min prior to the addition of insulin. After 5 h, cells were washed and MIR of the GTS was measured. B, adipocytes were incubated under similar conditions except 20 mM glucose was included in place of 2 mM glucosamine. Each bar represents the mean ± S.E. of three experiments. **, p < 0.01 using the Student's t test.](image)

![Fig. 7. Time-dependent decrease in glutamine:fructose-6-phosphate amidotransferase activity during treatment with actinomycin D (AD). Isolated adipocytes were suspended in HBSS (containing 20 mM glucose and 1 × amino acids) and treated with 200 nM actinomycin D. At the indicated times, cells were lysed, and enzymatic activity of GFAT in cytosol was determined as described previously (3). Each point represents the mean ± S.E. of three experiments. CX, cycloheximide.](image)
Prevention of Desensitization by mRNA Synthesis Inhibitors

FIG. 10. Effects of actinomycin D on the intracellular concentration of hexosamine products under desensitizing conditions. A, cells were suspended in HBSS and treated with 20 mM glucose, 25 ng/ml insulin, and 1 x amino acids in the absence or presence of 200 nM actinomycin D (AD) (A) or treated with 1 mM glucosamine and insulin with and without actinomycin D (B). After 90 min, all groups were lysed and hexosamine products were measured as described previously in the companion paper (3). Abbreviations are AD, actinomycin D; CTRL, control cells not exposed to insulin. Each bar represents the mean ± S.E. of three experiments. * p < 0.05; ** p < 0.001 using the Student’s t test.

The mammalian form of GFAT was extensively investigated in the early 1960s and 1970s, but there have been relatively few follow-up studies. Our findings that GFAT is an insulin-regulated enzyme (3) and linked to glucose-induced desensitization of the GTS in cultured adipocytes (18) raise new questions concerning the role of this enzyme in pathophysiological insulin-resistant states such as type II diabetes mellitus and obesity. However, before embarking on complex studies of altered physiological states, we felt it was important to gain a fundamental understanding of the mechanisms underlying regulation of GFAT. Thus, in the current paper, we explored the hypothesis that loss of GFAT activity under desensitizing conditions is mediated by transcriptional control at the gene level. One of our major objectives was to determine whether the turnover of GFAT in primary cultured rat adipocytes is sufficiently rapid to account for the temporal aspects of diminished GFAT activity. The experimental approach we chose was to block mRNA synthesis with either actinomycin D or DRB and to assess whether there was a progressive loss in GFAT activity.

When GFAT activity was measured in a crude cytosolic preparation at various times after exposing cells to actinomycin D, we observed a rapid and extensive loss of GFAT activity (t\text{1/2} of 45 min, >70% loss). These studies indicated that GFAT is a relatively short-lived protein and that continuous synthesis of mRNA is necessary to maintain GFAT levels. Additional studies revealed that recovery of GFAT activity does not occur in actinomycin D-treated cells after removal of this agent, consistent with the mechanism of actinomycin D action. In contrast, a full recovery was observed in cells pretreated for 2 h with 200 µM DRB to lower GFAT activity. This latter result is not unexpected, since previous (10) studies have shown that the inhibitory actions of DRB on mRNA synthesis in intact cells is fully reversible. On the basis of the concept that the steady-state level of a protein reflects a dynamic balance between synthesis and degradation, the finding that recovery of GFAT activity is fast (full recovery within 2 h) provides independent evidence that GFAT has a rapid turnover rate.

Based on the established importance of GFAT in mediating desensitization, we conducted studies to determine whether actinomycin D could prevent the induction of insulin resistance. Both actinomycin D and DRB rapidly and completely prevented desensitization of the GTS in a dose-dependent manner (ED_{50} values of 16 nM and 15 µM, respectively). Moreover, we found that the ability of DRB to block desensitization was fully reversible, whereas the action of actinomycin D was irreversible after a 30-min pretreatment period (for up to 18 h). These particular experiments indicate that functional integrity of the hexosamine desensitization pathway is maintained by a short-lived protein that is encoded for by a mRNA with a rapid turnover rate. The identity of this short-lived protein is most likely GFAT, the first and rate-limiting enzyme of the hexosamine biosynthesis pathway.

Supporting the idea that actinomycin D selectively affects an early step in the hexosamine-desensitization pathway (maintenance of GFAT levels) without altering the late events involved in the process of desensitization (all steps distal to the formation of glucosamine 6-phosphate) was the finding that actinomycin D failed to block glucosamine-induced desensitization of the GTS (Fig. 9). Since glucosamine readily enters cells, undergoes intracellular phosphorylation, and enters the hexosamine pathway at the level of glucosamine 6-phosphate (14–17), the only enzymatic step proximal to the point of glucosamine entry into the hexosamine pathway is amidation of fructose 6-phosphate by GFAT. Thus, under our experimental conditions, it appears that actinomycin D affects just GFAT and no other enzymes within this pathway. Consistent with this idea were the results of studies measuring the intracellular concentrations of hexosamine products (Fig. 10). When cells were treated for 90 min with glucose, insulin, and amino acids, the steady-state levels of hexosamine products were elevated >4-fold in agreement with the results of the preceding paper (3). Actinomycin D was able to effectively prevent this rise indicating that the activity of the rate-limiting enzyme of hexosamine biosynthesis (GFAT) was reduced. In contrast, the formation of hexosamine products from glucosamine was not impaired by actinomycin D, which we interpret to mean that enzymes distal to GFAT are unaltered by actinomycin D and fully capable of mediating the early steps involved in hexosamine biosynthesis.

DISCUSSION

The finding that actinomycin D blocks hexosamine desensitization in a dose-dependent manner (ED_{50} values of 16 nM and 15 µM, respectively) provides independent evidence that GFAT has a rapid turnover rate. The identity of this short-lived protein is most likely GFAT, the first and rate-limiting enzyme of the hexosamine biosynthesis pathway.
this $t_{1/2}$ value was in good agreement with the deinduction of enzyme synthesis observed when de novo synthesis of mRNA was blocked by inhibitors of mRNA processing, this suggested that insulin-induced loss of enzyme activity was due to altered mRNA levels (19). Subsequent studies confirmed this idea by revealing that mRNA synthesis inhibitors actually did cause a rapid decrease in phosphoenolpyruvate carboxykinase mRNA ($t_{1/2}$ of 40 min) and that the insulin-induced decrease in phosphoenolpyruvate carboxykinase synthesis was accompanied by a parallel change in the levels of mRNA encoding this enzyme (20–22). Although these experiments were in overall agreement, one potential problem inherent in the use of mRNA inhibitors is that the response under study may be mediated by nonspecific actions of these agents. This particular concern was minimized in our studies by showing that similar experimental results could be obtained using two inhibitors that block mRNA synthesis through completely different mechanisms (6–10, 23–27).

An interesting offshoot of the present study was the finding that inhibition of protein synthesis by cycloheximide failed to reduce the enzymatic activity of GFAT. This finding is consistent with our earlier study showing that cycloheximide was unable to block glucose-induced desensitization of the GTS (13) and indicates that cycloheximide failed to block desensitization, because GFAT levels were unaltered. However, an important and unanswered question revolving around cycloheximide action is why GFAT activity was not reduced given our current data indicating that GFAT has a rapid turnover rate. Possibly, cycloheximide may inhibit de novo biosynthesis of labile proteins involved in the turnover of GFAT or GFAT mRNA, or cycloheximide may impair degradation of various proteins, including enzymes such as GFAT. Along these lines, it has been shown previously that cycloheximide can cause superinduction of several proteins, such as c-Fos, c-Myc, and histone by preferentially stabilizing mRNA (28–30), whereas in other cases, cycloheximide can slow the turnover of selective proteins such as insulin receptors, estrogen receptors, and tyrosine amidotransferase (31–33). Clearly, additional studies will be required to resolve this apparent paradox.

Implicit in our model (Fig. 11) is the idea that formation of hexosamine products regulates GFAT activity by rapidly reducing the steady-state levels of GFAT mRNA and protein. Although this notion needs to be definitively demonstrated at the molecular level, all available evidence supports this idea. First, it appears unlikely that UDP-$N$-acytglycosamine or any other potential allosteric regulators are involved in the glucose-induced loss of GFAT activity. The evidence and arguments in support of this idea are presented in the companion paper (3) and in the introduction of this manuscript.

Second, the possibility that GFAT activity is modulated by phosphorylation/dephosphorylation reactions is diminished by the finding that insulin treatment (in the absence of glucose and glutamine) failed to alter enzyme activity. This finding is significant because that insulin receptor is a tyrosine-specific protein kinase (34) that can rapidly alter the phosphorylation state of a number of endogenous substrates and enzymes within minutes upon binding insulin (35). Since we observed changes in GFAT activity with a $t_{1/2}$ of 2 h, this finding argues against the idea that phosphorylation underlies changes in enzyme activity. Last, regulation at the gene level is supported by the results of the current study indicating that turnover of GFAT is in temporal agreement with the glucose-induced time course of GFAT regulation. Importantly, gene regulation of GFAT has precedent. In 1989 GFAT was cloned in yeast, and the gene that encodes this enzyme was found to be under rapid transcriptional control; within 15 min after adding a mating pheromone, gene expression was increased 2–3-fold (2). Considered together, the available evidence supports the idea that formation of a hexosamine product regulates GFAT activity at the pretranslational level (at least in part) by effecting GFAT gene transcription, mRNA stability, or both.

The list of enzymes known to be influenced by insulin through regulation of gene transcription or mRNA stability is rapidly growing, as is our understanding of insulin action at the molecular level. Of the genes that are induced by insulin, the most well studied are those encoding the enzymes pyruvate kinase, glyceraldehyde-3-phosphate dehydrogenase, fatty acid synthetase, malic enzyme, glucokinase, glucose-6-phosphate dehydrogenase, glucose synthetase, phosphoenolpyruvate carboxykinase, and tyrosine amidotransferase (37–42). For most of these enzymes, in vivo studies have shown that fasting and streptozotocin-induced diabetes result in a decrease in both mRNA levels and enzyme concentration in various tissues, whereas refeeding and insulin treatment of streptozotocin-treated rats restore mRNA levels and enzyme levels to control values. Although such studies are important in demonstrating that cellular enzymes are regulated by hormonal and dietary factors, one drawback of this in vivo approach is the difficulty in assessing whether insulin is acting directly or whether it is acting indirectly by affecting other hormones or altering systemic or cellular levels of metabolic substrates. A more direct approach, now commonly employed, is to use a cultured cell system to investigate the effects of insulin on enzyme protein, mRNA levels, and rates of gene transcription. In general, in vitro studies have confirmed that insulin plays a major role in inducing the synthesis of gene-regulated enzymes, and several investigators have advanced the field to the point where insulin-responsive elements on the 5' upstream region of the structural gene have been identified and mapped by deletional analysis.

Despite these advances, the mechanisms by which insulin regulates the transcription of various metabolic enzymes remains a mystery. For example, it is still unknown how insulin binding to cell surface receptors initiates a change in gene transcription. The major difficulties in addressing this question lie in our incomplete understanding of the molecular events underlying insulin action and in the fact that insulin exerts numerous and rapid pleiotropic effects on cellular function. For example, insulin binding rapidly triggers activation of insulin receptor tyrosine kinase activity, phosphorylation of numerous endogenous substrates of unknown function, activation/deactivation of various enzymes through phosphorylation-dephosphorylation reactions (43–45), and accelerates substrate uptake into cells (i.e. glucose and amino acids).
Prevention of Desensitization by mRNA Synthesis Inhibitors

Acknowledgment—We gratefully acknowledge the editorial assistance of Henri Kruse.

REFERENCES

1. Kletzien, R. F., Pariza, M. W., Becker, J. E., and Potter, V. R. (1975) Anal. Biochem. 68, 537-544
2. Watzke, G., and Tanner, W. (1989) J. Biol. Chem. 264, 8753-8758
3. Traxinger, R. R., and Marshall, S. (1991) J. Biol. Chem. 266, 10148-10154
4. Goldberg, I. H., and Reich, E. (1964) Fed. Proc. 23, 858-864
5. Dolecki, G. J., and Smith, L. D. (1979) Dev. Biol. 690, 219-236
6. Zandomeni, R., and Weinmann, R. (1984) J. Biol. Chem. 259, 14804-14811
7. Zandomeni, R., Zandomeni, M. C., Shugar, D., and Weinmann, R. (1986) J. Biol. Chem. 261, 3414-3419
8. Medico, F., Shugar, D., and Pinna, L. A. (1990) Eur. J. Biochem. 187, 89-94
9. Stevens, A., and Maupin, M. K. (1989) Biochem. Biophys. Res. Commun. 159, 508-515
10. Tamm, I., Hand, R., and Caligiuri, L. A. (1976) J. Cell Biol. 69, 229-240
11. Maher, F., Clark, E., and Harrison, L. C. (1989) Mol. Endocrinol. 3, 2128-2135
12. Kaestner, K. H., Christy, R. J., and Lane, M. D. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 251-255
13. Traxinger, R. R., and Marshall, S. (1989) J. Biol. Chem. 264, 8156-8163
14. Molnar, J., Robinson, G. B., and Winzler, R. J. (1964) J. Biol. Chem. 239, 3157-3162
15. DelGiacco, R., and Maley, F. (1964) J. Biol. Chem. 239, 2400-2402
16. Kornfeld, S., and Ginsburg, V. (1966) Exp. Cell Res. 41, 592-600
17. Bekesi, J. G., and Winzler, R. J. (1969) J. Biol. Chem. 244, 5663-5668
18. Marshall, S., Bacote, V., and Traxinger, R. R. (1991) J. Biol. Chem. 266, 3207-3210
19. Tilghman, S. M., Hanson, R. W., Reshef, L., Hopwood, M. F., and Ballard, F. J. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 1304-1308
20. Kioussis, D., Reshef, L., Cohen, H., Tilghman, S. M., Iynedjian, P. B., Ballard, F. J., and Hanson, R. W. (1978) J. Biol. Chem. 253, 4327-4332
21. Hod, Y., and Hanson, R. W. (1988) J. Biol. Chem. 263, 7747-7752
22. Nelson, K., Cimbala, M. A., and Hanson, R. W. (1980) J. Biol. Chem. 255, 8800-8816
23. Sehgal, P. B., Darnell, J. E., Jr., and Tamm, I. (1976) Cell 9, 473-480
24. Tamm, I., and Kikuchi, T. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 5750-5754
25. Fraser, N. W., Sehgal, P. B., and Darnell, J. E. (1978) Nature 272, 590-593
26. Laub, O., Jakohovits, E. B., and Aioni, Y. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 3297-3301
27. Zandomeni, R., Mittleman, B., Bunick, D., Ackerman, S., and Weinmann, R. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 3167-3170
28. Kelly, K., Cochran, B. H., Stiles, C. D., and Leder, P. (1983) Cell 35, 603-610
29. Krujver, W., Cooper, J. A., Hunter, T., and Verma, I. M. (1984) Nature 312, 711-716
30. Stimm, E., Groppi, V. E., and Coffino, P. (1984) Mol. Cell. Biol. 4, 2082-2090
31. Knutson, V. P., Ronnett, G. V., and Lane, M. D. (1985) J. Biol. Chem. 260, 14180-14188
32. Campen, C. A., and Gorski, J. (1986) Endocrinology 119, 1454-1461
33. Kenney, F. T. (1967) Science 156, 525-528
34. Kahn, C. R., and White, M. P. (1988) J. Clin. Invest. 82, 1151-1156
35. Shoelson, S. E., and Kahn, C. R. (1989) in Molecular and Cellular Biology of Diabetes Mellitus: Insulin Action (Draznin, B., Melmed, S., and LeRoith, D., eds) pp. 23-24, Allan R. Liss Inc., New York
36. Deleted in proof
37. Goodridge, A. G. (1990) FASEB J. 4, 3099-3110
38. Granner, D., and Pilkis, S. (1990) J. Biol. Chem. 265, 10173-10176
39. Meisler, M. H., and Howard, G. (1989) Annu. Rev. Physiol. 51, 701-714
40. Messina, J. L. (1990) in Handbook of Experimental Pharmacology (Cuatrecasas, P., and Jacobs, S., eds) pp. 399-419, Springer-Verlag, Heidelberg, Federal Republic of Germany
41. Miller, R. E., and Burns, D. M. (1985) Curr. Top. Cell. Regul. 26, 65-78
42. Moore, P. S., and Koontz, J. W. (1989) Mol. Endocrinol. 3, 1724-1732
43. Czech, M. P., Massague, J., Seals, J. R., and Yu, K-T. (1984) in Biochemical Action of Hormones (Litwack, G., ed) pp. 93-125, Academic Press Inc., New York
44. Avruch, J., Nemenoff, R. A., Pierce, M., Kwok, Y. C., and Blackshear, P. J. (1985) in Molecular Basis of Insulin Action (Czech, M. P., ed) pp. 263-296, Plenum Press, New York
45. Czech, M. P., Klarlund, J. K., Yaglaloff, K. A., Bradford, A. P., and Lewis, R. E. (1988) J. Biol. Chem. 263, 11017-11020