Inhibition and Stimulation of c-myc Gene Transcription by Insulin in Rat Hepatoma Cells

INSULIN ALTERS THE INTRAGENIC PAUSING OF c-myc TRANSCRIPTION* (Received for publication, May 21, 1991)

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One of insulin’s least studied actions is its ability to induce DNA synthesis and cell division. In rat H4IIE hepatoma cells insulin, acting through its own receptor, stimulates cell division. However, little is known about the molecular mechanisms involved in this effect. The proto-oncogene c-myc is a cellular gene which when expressed at abnormal levels is often associated with the process of tumorigenesis. Expression of the normal cellular c-myc gene may be necessary for growth factor-induced cell cycling. In the present work, insulin was shown to regulate cellular accumulation and transcription of the c-myc gene in rat hepatoma cells. The control of c-myc by insulin was complex, with an initial-induced decrease in c-myc transcription to 50% of control values at 15 and 30 min. This was followed by an increase in transcription of about 3-fold by 60-120 min. Similar to the initial inhibitory effect of insulin, the protein synthesis inhibitors cycloheximide or anisomycin decreased c-myc transcription. However, there was no secondary induction of c-myc transcription by protein synthesis inhibitors. The effects of both insulin and protein synthesis inhibitors were shown to be through alterations in intragenic pausing of transcription of the sense mRNA, not through changes in initiation of transcription.

Insulin is important for the normal growth and development of mammals. When this hormone is deficient, or when cells are resistant to the actions of this hormone, normal growth is impaired (1-3). The ability of insulin to induce DNA synthesis was shown in various cell types in vitro including human fibroblasts, mouse 3T3 fibroblasts, mouse preadipocytes, hamster kidney cells, rat liver cells, and rat and human hepatoma cells (4-10).

Little is known about the mechanisms by which insulin regulates cell division. It is assumed that a chain of cellular events involving controlled expression of specific genes and posttranslational modifications of specific proteins is necessary for cell proliferation. Several growth factor-regulated genes are considered components of this chain of events leading to cell division or differentiation. One such gene is the proto-oncogene c-myc, a cellular gene whose alteration by mutation or deregulation has been implicated in the process of tumorigenesis (11-14).

Expression of the normal cellular c-myc gene is believed to be involved in the intracellular signaling mechanism leading to an increase in DNA synthesis and cell division. A rapid induction in the transcription of the c-myc gene was observed following growth factor or mitogen addition to quiescent fibroblasts, lymphocytes, or muscle cells (15-19). Enhanced expression of c-myc has been observed in several neoplasms and this gene alters cellular transformation or differentiation when transfected or transduced (via retroviruses) into cells (20-23). When the c-myc gene was transfected into cells alone or cotransfected with the v-Ha-ras gene, cells lost their dependency on external influences, such as growth factors, for cell division (24-26). Thus, current evidence suggests an important role of the c-myc protein product in the control of cell proliferation.

The precise role and mode of action of the c-myc protein in cellular growth is still under study. However, insulin and growth factors such as platelet-derived growth factor and insulin-like growth factor I can increase c-myc mRNA levels in various cell lines (16, 19, 27). Thus, the ability of insulin to directly or indirectly regulate the expression of the c-myc gene is important in understanding insulin’s role as a growth factor. In this report we show that in rat hepatoma cells insulin regulated transcription of the c-myc gene in a complex manner with rapid inhibition and then a later induction of transcription. Like insulin, addition of protein synthesis inhibitors repressed c-myc transcription, but did not result in the secondary stimulation of c-myc gene expression. Transcriptional activity of the c-myc gene is higher in exon 1 than in the remainder of the c-myc gene. This indicates that transcription pauses near the 3’ end of exon 1 (28). Both insulin and protein synthesis inhibitors were shown to alter whole gene transcription rates by regulating intragenic pausing of transcription of the sense strand of the c-myc gene.

EXPERIMENTAL PROCEDURES

Materials—Sodium insulin was a gift from Dr. Ronald Chance (Eli Lilly Co., Indianapolis, IN). Swim’s 77 media, fetal bovine serum, and horse serum were purchased from GIBCO. Epidermal growth factor (EGF), fibroblast growth factor (FGF), vanadyl-ribonucleoside complex, and proteinase K were purchased from Bethesda Research Laboratories. RNasin was purchased from Promega (Madison, WI). DNase I and RNase T1 were purchased from Worthington/Cappel.

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1 Portions of this paper (including parts of “Experimental Procedures” and “Results,” Table 2, and Figs. 5-7) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

2 The abbreviations used are: EGF, epidermal growth factor; FGF, fibroblast growth factor; kb, kilobase(s).
Biochemicals (Freehold, NJ), [α-32P]dCTP (3200 Ci/mM), [α-32P] UTP (3000 Ci/mM), and [3H]leucine (110 Ci/mM) were purchased from ICN. All other reagents were purchased from Sigma.

**Cell Culture**—H4IIIE hepatoma cells (H4; American Type Culture Collection (Rockville, MD)) were grown in 10-cm tissue culture plates in 20 ml medium with 5% fetal bovine serum and 5% horse serum in a 5% CO2 incubator at 85% humidity. Serum was withdrawn for 20-24 h prior to experiments when the cells were approximately 60% confluent (29).

**Isolation and Quantification of Cytoplasmic c-myc mRNA**—RNA was isolated by a modification of the method of Favaloro et al. (30) as described (31). Briefly, cells were washed in Dulbecco's salt solution, scraped, and resuspended in a lysis buffer containing 10 mM Tris-HCl (pH 8.0), 140 mM sodium chloride, 1.5 mM magnesium chloride 0.5% NP-40, and 10 mM vanadyl-ribonucleosamide complex. The nuclei were pelleted and the supernatant was diluted with one volume of 200 mM Tris-HCl pH 7.5, 400 mM sodium chloride, 2 mM dodecyl sulfate, and 25 mM EDTA, extracted with phenol/chloroform (1:1), and ethanol precipitated. Northern gels and transfers were performed using 10 μg of cytoplasmic RNA as previously described (31). Hybridization to an excess of nick-translated pBR322 containing a BamHI/HindIII insert of the mouse c-myc cDNA (ATCC; Catalog 41029) was performed as previously described (29). Nitrocellulose filters were washed extensively and exposed to x-ray film. Densitometric scans were made of resultant autoradiograms. The control for each experiment was arbitrarily set to unity and the densities of experimental samples were compared to that of controls.

**Isolation of Nuclei and the Measurement of Transcription**—Nuclear run-on assays were performed (32) to measure the elongation of transcripts in control or treated cells. In brief, nuclei were prepared from approximately 1 × 107 cells by homogenization in 0.3 M sucrose, 2 mM magnesium acetate, 3M calcium chloride, 10 mM Tris-HCl, pH 8.0, 2.75 mM dithiorthreitol, and 1 unit/ml RNasin. Cell diution buffer (2.0 M sucrose, 5 mM magnesium acetate, 10 mM Tris-HCl, pH 8.0, 2.75 mM dithiorthreitol, 1 unit/ml RNasin) was added (2.1 v/v). This mixture was layered over cell diution buffer and nuclei were isolated by centrifugation at 30,000 x g for 45 min.

Nuclei were resuspended and incubated in a buffer containing 25 mM Tris-HCl, pH 8.0, 5 mM magnesium acetate, 12.5% glycerol, 100 mM ammonium sulfate, 1 mM ATP, 0.4 mM GTP, and GTP, 5 mM dithiorthreitol, 100 units/ml RNasin, 0.05 mM EDTA, and 100 μCi of [α-32P]UTP. Incubations were for 45 min at 26 °C and then ended by Proteinase K followed by addition of calcium chloride and DNase I. RNA was isolated by sequential sodium perchlorate/ethanol precipitation (33), phenol extraction, trichloroacetic acid precipitation, and sodium acetate/ethanol precipitation (32, 34).

Labeled RNA was incubated with nitrocellulose previously dotted with 2 μg of either pBR322 plasmid DNA, or pBR322 containing a chicken β-tubulin cDNA insert (35), or pBR322 containing a double-stranded c-myc cDNA insert. The transcription rates of several other oncogenes, were also studied (32) to measure the elongation of transcripts (29). The number of counts per minute (cpm) of radioactively labeled RNA added to the nitrocellulose filters varied between 4 × 106 and 23 × 106 in experiments performed on different days. However, within each experiment, the number of counts in the control and experimental groups were identical. The filters were incubated with radioactively labeled RNA for 65-70 h at 65 °C and then washed in 0.3 M sodium chloride, 0.03 M sodium citrate and treated with RNase A and RNase T1. Following autoradiography, the extent of hybridization of labeled RNAs to cDNA probes were quantified by densitometric scanning of the autoradiograms. The densitometric signal of the experimental groups were expressed as a percentage of control (untreated) group of each experiment.

For study of intragenic pausing and antisense transcription, single-stranded M13 probes were obtained (28) and used in transcriptional studies as described above except that 200 μCi of [α-32P]UTP was used per experimental group. Two probes (B36 and 11BH) were complementary to sense transcripts and therefore measured transcription in the sense direction. The B36 probe, a BamHI-BgIII 0.5 kb fragment spans the majority of exon 1. The 11BH (BamHI-HindIII) probe spans 3.3 kb which includes the 3' half of exon 1, all of exon 2 and intron 2, and most of exon 3. Thus, the B36 probe measures transcription rates of exon 1 and the 11BH probe measures transcription only from sequences downstream of exon 1. For antisense transcription, two probes complementary to antisense transcripts (S10–20 and P30) were used. The S10–20 (Setl-BamHI) 0.9 kb probe spans the 3' end of exon 1 and the 5' half of intron 1. The 1.2-kb P30 probe is made up of the 3' end of exon 2 and most of exon 3 (28). These two probes therefore measure antisense transcription from two separate regions of the c-myc gene. The densitometric signal of the experimental groups were again expressed as a percentage of the control (untreated) group of each experiment. In addition, as described by Nepveu and Marcu (28), the raw densitometric output obtained for the two sense-strand probes (B36 and 11BH) were compared in a ratio of the early gene (B36; exon 1) and late gene (11BH; exons 2 and 3 and introns 1 and 2) transcription rates. Statistical analysis was performed using Student's t test.

**Results**

**Regulation of c-myc mRNA by Insulin**—Insulin increased the cytoplasmic concentration of c-myc mRNA in a time- and dose-dependent manner. As shown in Fig. 1, insulin stimulated the accumulation of c-myc mRNA in H4 cells after 60 min (lanes b and e) and 120 min (lanes c and f) compared to cells not treated with insulin (lanes a and d). When quantified by densitometry, insulin stimulated an average 8-9-fold increase (range for seven experiments: 5.9-13.5-fold increase) in c-myc mRNA by 60 min. These experiments were performed at 5 mM insulin. Higher concentrations of insulin were no more effective and lower concentrations gave a proportionally lesser effect (data not shown).

**Transcriptional Effects of Insulin on c-myc and Other Oncogenes**—Several control experiments were performed to confirm the linearity of our nuclear run-off assays and have been presented previously (32). Insulin regulated the transcription of the c-myc gene in a complex manner. Insulin treatment resulted in a decrease of c-myc transcription within 15 min of insulin treatment (5 mM; Fig. 2, A and B). This 40-50% reduction was maintained for approximately 30 min. However, by 60 min the effects of insulin had reversed and there was a 230% increase in transcription of the c-myc gene (Fig. 2). The induction of c-myc mRNA by insulin further increased to 320% over nonstimulated levels by 120 min. The effect of insulin on transcription of the c-myc gene was also determined at 24 h following insulin addition and was found to be comparable to transcription rates in basal, untreated cells (data not shown).

![Fig. 1. Effect of insulin on the cytoplasmic concentration of c-myc mRNA.](image-url)
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not shown). Insulin had no effect on the transcription of the β-tubulin gene (Fig. 2B).

The ability of increasing concentrations of insulin to inhibit the transcription of c-myc mRNA is shown in Fig. 3. A minimum concentration of 0.5 nM insulin was necessary to consistently inhibit transcription of the c-myc gene to 54% of control values although in some experiments small decreases in transcription were observed at lower insulin concentrations. Insulin at 5 nM or higher concentrations resulted in reductions to 40–45% of the control transcription rate.

The effects of insulin on the transcription of several other oncogenes were studied in these experiments and insulin was found to have no effects on the transcription of the sis, p53, erb-b, and myb oncogenes (data not shown).

Regulation of the c-myc and Other Oncogenes by Serum and Other Growth Factors—Fetal bovine serum is known to stimulate cell division in H4 cells (5). Since the c-myc gene may be a growth-related gene, serum regulation of c-myc transcription was studied. Addition of 5 or 10% fetal bovine serum to 24-h serum-starved H4 cells for 30 min (Fig. 4, Ser lanes: b and c) or 60 min (data not shown) resulted in a 250–300% induction of c-myc gene transcription. Unlike insulin, no reduction in transcription of the c-myc gene was found following addition of serum for 30 min. Induction of c-myc transcription by serum returned towards basal levels by 120 min (data not shown). Thus, the effects of serum contrasted greatly with those of insulin since insulin inhibited c-myc transcription at 30 min and stimulated transcription at 120 min (Fig. 2B).

In separate experiments, two other growth factors, EGF and FGF, were ineffective in altering the transcription rate of the c-myc gene when added to H4 cells at concentrations of 5 and 100 ng/ml, respectively (Fig. 4, lanes EGF-FF: b and c). Likewise, transcription of the sis, p53, ros, erb-b, and myb oncogenes did not respond to the addition of serum, EGF, or FGF under the conditions tested (data not shown).

Role of Intragenic Attenuation in the Action of Insulin on Transcription of the c-myc Gene—Single-stranded, region-specific sense and antisense probes were used to study transcription of the c-myc gene in response to insulin. In the antisense direction, the two regions tested exhibited low but significant levels of transcription. This transcription was approximately 10-fold less than in the two regions examined for sense transcription. Furthermore, there were no significant changes in antisense transcription following insulin ad-
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<table>
<thead>
<tr>
<th>DNA probe</th>
<th>Orientation</th>
<th>Location in c-myc</th>
<th>Time/treatment</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control, 0 min</td>
</tr>
<tr>
<td>B36</td>
<td>+</td>
<td>Exon 1</td>
<td>100%</td>
</tr>
<tr>
<td>11BH</td>
<td>+</td>
<td>Exons 2-3 and introns 1-2</td>
<td>100%</td>
</tr>
<tr>
<td>S10-20</td>
<td>-</td>
<td>Intron 1</td>
<td>100%</td>
</tr>
<tr>
<td>F30</td>
<td>-</td>
<td>Exons 2-3</td>
<td>100%</td>
</tr>
<tr>
<td>Ratio of B36/11BH</td>
<td></td>
<td></td>
<td>0.37</td>
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*Statistically different from control, p < 0.05; analyzed by a Student’s two-tailed t test.

The use of single-stranded sense probes produced results agreeing with an intragenic pausing at the 3′ end of the first exon (28). A strong transcriptional signal was obtained from exon 1 (B36 probe) and this transcription rate did not change significantly upon insulin treatment (Table I). Transcription rates measured using the downstream probe (11BH) resulted in a very different profile. Following 15 min of insulin addition, measured transcription rates were significantly decreased (Table I). This was equivalent to the data found using the double-stranded probe (see Figs. 2 and 5). There also was a significant increase in transcription rates measured in this locus following 120 min of insulin addition. Again, this was in agreement with data presented earlier (Fig. 2, A and B). Together, these results indicate that transcription of the c-myc gene through exon 1 remains high during all tested conditions, and it was the degree of transcriptional pausing that was regulated. When transcription was induced (insulin, 120 min), attenuation of transcription was reduced. Conversely, when transcription of c-myc was inhibited, there was an increase in transcriptional attenuation at the 3′ end of the first exon. This hypothesis was supported by the ratio of early and late transcriptional signals (B36/11BH). Compared to control cells, the ratio increased following 15 min of insulin, indicating a relative decrease in late gene transcription (Table I). Following 120 min of insulin addition, the ratio decreased markedly, indicating a relative increase in late gene transcription.

DISCUSSION

The exact role of the c-myc protein is being studied intensively. It is known to be localized to the nucleus and has been reported to be associated with the nuclear matrix, small nuclear ribonucleoproteins and DNA replication sites (38–42). The c-myc protein may act as a regulatory protein for several genes, including itself (42). When the c-myc protein was microinjected into nuclei, it substituted for growth factors in the induction of cell division (43). When the c-myc gene was introduced into cells under the control of either retroviral, heat-shock or metallothionein promoters, there was induction of growth-related genes (44, 45) and cells lost their dependency on, or became hyperresponsive to growth factors (24, 26, 44, 46, 47). In other work, transfection of the c-myc gene inhibited differentiation of erythroleukemia cells (21, 26, 48) and stimulated transformation of embryonic fibroblasts (20). c-myc is regulated at various points in the cell cycle (49) and in primary rat hepatocytes is elevated when cells are actively dividing (50). An increase in intracellular cAMP resulted in a decrease in c-myc mRNA and cell cycle arrest in human promyelocytic cells and foreskin fibroblasts (51, 52). Conversely, an increase in cAMP resulted in an increase in c-myc mRNA and an increase in cell division in rat H4 hepatoma cells (53).

Insulin induces DNA synthesis and cell division in a variety of cell types in culture including human mammary tumor cells, rat hepatocytes and rat and human hepatoma cells (1, 10, 54). It has been difficult to determine if insulin’s growth stimulatory effects were due solely to its interaction with the insulin receptor (1). Current evidence suggests that insulin’s effect on cell division in H4 hepatoma cells was through interaction with the insulin receptor and not through interactions with IGF-I or IGF-II receptors (55–57). This is in agreement with the present work demonstrating that concentrations of insulin well within the physiologic range regulated the cellular accumulation of c-myc mRNA. However, since c-myc mRNA stability can be regulated (58, 59), it was not known whether insulin could modulate transcription of the c-myc gene. The present study is the first to demonstrate regulation of c-myc transcription and to our knowledge, this is the first instance of insulin having a rapid biphasic action on the transcription of a gene. However, even the secondary induction of c-myc transcription by insulin was not sufficient to account for the observed increase in cellular concentrations of c-myc mRNA. This would suggest that in addition to its biphasic transcriptional effects, insulin could also regulate a posttranscriptional process, possibly involving the stability of c-myc mRNA (58, 59).

Insulin’s regulation of the c-myc gene is not mimicked by the addition of serum. Serum does not have a biphasic effect on the transcription of c-myc and the effects of serum had returned to control levels by 120 min, when insulin’s effects were at their peak. Thus, serum is not simply supplying a source of insulin to the cells but is having a different effect, possibly by supplying other growth factors or agents that regulate the c-myc gene in a way distinct from insulin. These data indicate that the c-myc gene can be regulated differentially in H4 cells and that insulin’s effects were specific for insulin. In addition, serum is not simply supplying either EGF or FGF factor since these growth factors, when added individ-
usually, were ineffective in altering c-myc transcription.

There are two mechanisms for controlling the transcription of a gene. One is modifying the rate of initiation, a step commonly modified by hormones. The second, and to date more rare mechanism for hormonal regulation, is altering elongation of a transcriptionally initiated gene. In exon 1 of the mouse and human c-myc genes, elongation of the transcript is normally inhibited (28, 60-63). This intragenic pausing can be strengthened to decrease transcription of the remainder of the gene or be weakened to allow increased transcription of the entire gene (61, 64). The use of single-stranded, region-specific probes in our transcription studies unequivocally demonstrated that transcription of the c-myc gene through exon 1 remains high in rat H4 hepatoma cells under the conditions tested and it was the degree of transcriptional attenuation, or pausing, that was regulated. When transcription was induced by insulin, attenuation of transcription was reduced. Conversely, when transcription of c-myc was inhibited by insulin, there was an increase in transcriptional attenuation at the 3' end of the first exon. This hypothesis was supported by the ratio of early and late transcriptional signals. This is the first instance demonstrating a regulation of intragenic pausing of a gene by insulin.

The use of single-stranded probes were also useful to verify that antisense transcription was in fact occurring in H4 cells. The level of antisense transcription was low and there was no regulation by insulin. Thus, the biphasic changes in transcription in response to insulin found using the double-stranded probes was due to alterations in transcription of the sense strand of the gene.

In summary, the data presented here suggest multiple effects of insulin in the regulation of the c-myc gene whose product may play a role in cell division. An unusual finding in the present work is that one agent, insulin, regulates the transcription of the entire gene (61, 64). The use of single-stranded probes were also useful to verify that antisense transcription was in fact occurring in H4 cells. The level of antisense transcription was low and there was no regulation by insulin. Thus, the biphasic changes in transcription in response to insulin found using the double-stranded probes was due to alterations in transcription of the sense strand of the gene.

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REFERENCES

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**Experimental Procedures**

**H-4 Leucine Incorporation into Proteins**. Cells were deprived of serum for 24 h and then exposed to the protein synthesis inhibitors cycloheximide (Cyc) or anisomycin (Anis) for 60 min. The cells were then exposed to the protein synthesis inhibitors for 24 h. Following a 30-min incubation with cycloheximide or anisomycin, the cells were washed and exposed to serum-free medium. The cells were then incubated for 24 h with or without the protein synthesis inhibitors.

**Results**

**Transcriptional Effects of Protein Synthesis Inhibitors on the c-myc and Other Oncogenes**

Protein synthesis inhibitors have been used to study the transcription of many different genes. For example, protein synthesis inhibitors have been used to study the transcription of the c-myc gene. When the transcription of the c-myc gene is inhibited, the expression of the c-myc gene is reduced. The results show that the transcription of the c-myc gene is reduced when the cells are treated with protein synthesis inhibitors. The results also show that the transcription of the c-myc gene is increased when the cells are treated with protein synthesis inhibitors.

**Combination of Insulin and Protein Synthesis Inhibitors on Transcription of the c-myc Gene**

Since protein synthesis inhibitors decrease transcription of the c-myc gene and insulin transiently decreases transcription of the c-myc gene, we asked whether insulin and protein synthesis inhibitors together would have an additive effect in reducing c-myc transcription. As shown previously, when insulin was added to H4 cells for 15 or 30 min, there was a decrease in c-myc transcription. When Cyc (50 μM) or Anis (20 μM) were added for 60 min, there was a 60–70% decrease in c-myc transcription. The addition of insulin for the last 30 min (70) or the last 15 min (St) showed a 60–80% decrease in Cya or Anis alone. The addition of Cyc or Anis (alone or added) showed no effect on the transcription of the c-myc gene.

**Role of Intracellular Uptake in the Activation of Protein Synthesis Inhibitors on Transcription of the c-myc Gene**

Single-stranded, region-specific antisense and sense probes were used to study the transcription of the c-myc gene in response to protein synthesis inhibitors. In H4 cells, there was an increase in transcription of the c-myc gene at 3 min following 30 min of anisomycin treatment (28). There was no significant change in c-myc transcription in response to the P30 probe, which measures transcription from exon 4 and 5.

**Discussion**

The action of the two protein synthesis inhibitors on the transcription of the c-myc gene suggests that this gene is under the control of a rapid turnover regulatory protein. The results show that the transcription of the c-myc gene is reduced when the cells are treated with protein synthesis inhibitors. For example, the transcription of the c-myc gene is reduced when the cells are treated with protein synthesis inhibitors. It is possible that the transcription of the c-myc gene is increased when the cells are treated with protein synthesis inhibitors. This implies that the insulin’s action, like that of the protein synthesis inhibitors, may be to reduce the amount or activity of a stimulatory protein.

**TABLE 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% of Control</th>
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<tbody>
<tr>
<td>Cyc 1 μM</td>
<td>69.000 18.3</td>
</tr>
<tr>
<td>* 10 μM</td>
<td>63.000 3.1</td>
</tr>
<tr>
<td>* 50 μM</td>
<td>13.000 4.1</td>
</tr>
<tr>
<td>Anis 1 μM</td>
<td>23.000 7.2</td>
</tr>
<tr>
<td>* 10 μM</td>
<td>9.300 2.8</td>
</tr>
<tr>
<td>* 30 μM</td>
<td>9.100 2.8</td>
</tr>
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</table>

* * Cells were treated with cycloheximide (Cyc) or anisomycin (Anis) for 60 min and then H-4 cells were added for 120 min as described in Methods. Results are the mean of 2 experiments performed in triplicate.
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Figure 5. TIME COURSE OF PROTEIN SYNTHESIS INHIBITORS ON TRANSCRIPTION OF THE C-MYC GENE.
H4 cells were deprived of serum for 24 h and treated with cycloheximide (Cyc, A); 50 
μg/ml or anisomycin (Anis, B; 30 μM) for the designated times. Nuclei were isolated and transcription was measured as described in the legend for Fig. 2. The autoradiographs were scanned and the results are the means of 3-4 separate experiments for Cyc (A) and 2-5 experiments for Anis (B). The vertical lines indicate ± SE.

Figure 6. DOSE RESPONSE OF CYCLOHEXIMIDE AND ANISOMYCIN ON TRANSCRIPTION OF THE C-MYC GENE.
H4 cells were deprived of serum for 24 h and treated with Cyc or Anis at the indicated concentrations for 60 min. Nuclei were isolated and transcription was measured as described in the legend for Fig. 2. The results depicted are the means of autoradiographs of four separate experiments for Cyc and Anis. The vertical lines indicate ± SE.

Figure 7. EFFECTS OF ADDITION OF BOTH INSULIN AND EITHER CYCLOHEXIMIDE OR ANISOMYCIN ON TRANSCRIPTION OF THE C-MYC GENE.
H4 cells were deprived of serum for 24 h and treated with insulin (Ins, 5 x 10^-6) for 30 min, Cyc (A, 50 μg/ml) for 60 min, Anis (B, 30 μM) for 60 min or a combination of a protein synthesis inhibitor with insulin added for the last 30 min. Nuclei were isolated and transcription was measured as described in the legend for Fig. 2. The results are the means of 4 separate experiments for Cyc (A) and 3 separate experiments for Anis (B).