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# Newly synthesized RNA: Simultaneous measurement in intact cells of transcription rates and RNA stability of insulin-like growth factor I, actin, and albumin in growth hormone-stimulated hepatocytes

**(4-thiouridine/cultured hepatocytes/mercurated agarose affinity chromatography)**

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ABSTRACT **The levels of several RNA transcripts in cultured hepatocytes are regulated by transcriptional and post-transcriptional mechanisms and are affected by growth hormone and insulin. We assessed the effects of these hormones on transcription rates and the stability of insulin-like growth factor I, actin, and albumin transcripts in intact cells of primary cultures of rat hepatocytes by analyzing thiol-labeled, newly synthesized RNA isolated by mercurated agarose affinity chromatography. The application of this concept to the measurement of transcript stability is presented in detail. The data indicate that growth hormone stimulates the transcription rates of insulin-like growth factor I, actin, and albumin genes. The stability of all three transcripts, particularly albumin, appears to be lower in growth hormone-containing medium than it is in insulin-containing medium. The experiments indicate that the rates of transcription and/or degradation of albumin mRNA are influenced by hormonal treatment. However, the cells maintain roughly constant albumin transcript levels independent of hormone treatment by compensatory changes in the rates of transcription and degradation.**

We reported previously (1) that growth hormone (GH) and insulin stimulated the accumulation of insulin-like growth factor I (IGF-I) and actin transcripts in rat hepatocyte primary cultures. By analyzing thiol-labeled, newly synthesized RNA isolated by affinity chromatography with mercurated agarose, we were able to separate the newly synthesized RNA and showed that insulin and GH exerted their effects on IGF-I transcript levels by stimulating transcription. Since it is known that a related hormone, prolactin, has profound effects on the casein mRNA transcript half-life as well as on casein transcription (2), we wished to determine whether GH also affected IGF-I transcript half-life. However, when traditional methodology employing actinomycin D was applied to primary hepatocyte cultures, it was observed that IGF-I transcript levels actually increased in the presence of inhibitor concentrations sufficient to suppress [$^3$H]uridine incorporation by 98%. Similar results have been reported for IGF-I transcripts in U937 cells (3) and rat C6 glial cells (4). This phenomenon was also observed for albumin but not for $\beta$-actin RNA transcripts. Thus, the approach of using transcriptional inhibitors to estimate transcript half-lives is inappropriate for this particular system. In addition, IGF-I transcript levels are not sufficiently abundant to make pulse–chase studies readily feasible.

In this report we show that analysis of thiolated, newly synthesized RNA, in addition to yielding data on relative transcription rates for specific transcripts under different hormone treatments, can provide estimates of transcript half-life, which are not subject to the same limitations as studies employing transcriptional inhibitors or pulse–chase methods. Moreover, our results indicate that GH increases transcription of actin, albumin, and IGF-I.

## MATERIALS AND METHODS

The preparation of primary hepatocyte cultures, isolation of RNA, RNA transfer blots, and clone sources were as described (1), except that hepatocytes were obtained by perfusion via the portal vein. Newly synthesized RNA was prepared according to the protocol of Woodford *et al.* (5). Briefly, 4-thiouridine (Sigma) was dissolved immediately before use in sterile water that had been purged with $N_2$. It was added at a final concentration of 100 $\mu$M to prewarmed culture medium [serum-free HCD medium (6) lacking dimethyl sulfoxide, with the indicated hormonal supplements]. The medium contained, in addition, [$^3$H]uridine at 5 $\mu$Ci/ml (1 Ci = 37 GBq). Basal medium is defined as HCD containing hydrocortisone (10 nM) and triiodothyronine (1 nM). Hormone supplements, where used, were porcine insulin (Sigma) at 500 ng/ml and bovine growth hormone (USDA-bGH-B-1; obtained from the National Hormone and Pituitary Program and distributed by the National Institute of Diabetes, Digestive, and Kidney Diseases) at 500 ng/ml. Hepatocytes were exposed to the thiol and tritium labels by changing medium. After an hour of incubation, the medium was aspirated and cells were lysed on the plates by addition of guanidine thiocyanate (7). The cell lysate was frozen in dry ice/ethanol and stored at −70° until further analysis. Control experiments established that [$^3$H]uridine and presumably 4-thiouridine entered RNA within a few minutes after medium change. The specific activity of total RNA labeled by changing medium in this fashion was identical to that obtained by introducing [$^3$H]uridine directly into the cultures.

Prior to performing this study, a number of controls on the thiol-labeling procedure were carried out. No binding of RNA to the affinity matrix was observed when 6-thioguanosine was used as the thiolated precursor, at any concentration suggested by Woodford *et al.* (5). When 4-thiouridine was used as the precursor, the recovery of labeled RNA applied to the mercurated agarose was proportional to the 4-thiouridine concentration (see table 2 in ref. 1). A 1-hr incubation with 100 $\mu$M 4-thiouridine was chosen because (*i*) the pattern of total accumulation of the three transcripts studied in the four different incubation conditions used was the same in the presence or absence of a 1-hr exposure to 100 $\mu$M 4-thiouridine, indicating that it was not toxic by that criterion; (*ii*) these conditions provided efficient enough labeling to bind 80–90% of the thiol- and $^3$H-labeled newly synthesized RNA

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Abbreviations: GH, growth hormone; IGF-I, insulin-like growth factor I.

to Affi-Gel 501 without the use of possibly toxic agents to deplete endogenous uridine pools (2); and (*iii*) although some labeled RNA was lost during the wash steps following application of RNA to Affi-Gel 501, these conditions allowed 47% recovery of labeled RNA (see table 2 in ref. 1) in the final eluate with a specific activity 5- to 10-fold higher than RNA before fractionation, indicating specific binding. Binding of RNA to the affinity matrix was done at 4°C in the dark, since 4-thiouridine is photoactivatable (8).

The experiments were repeated three times (three different rats), of which data are presented for two. The third employed a different labeling protocol for 4-thiouridine and was not analyzed for actin and albumin transcripts; however, the same trends for IGF-I transcripts were observed. Since interpretation of the data relies heavily on criteria met within individual experiments, data from individual experiments are presented separately.

## RESULTS

To assess the effects of insulin and GH on transcription of IGF-I, actin, and albumin, the following experiment was performed. Hepatocytes were plated in serum-free, insulin-containing medium. After cell attachment, the medium was replaced with basal medium lacking serum and supplemented only with hydrocortisone and triiodothyronine. After 24 hr, the plates were divided into four groups. Group 1 received no additional supplement. Group 2 received insulin alone (500 ng/ml). Group 3 received GH alone (500 ng/ml), and group 4 received insulin and GH. After a 23-hr incubation, the medium was replaced with medium containing the same hormonal supplements and, in addition, 100 $\mu$M 4-thiouridine and [$^3$H]uridine (5 $\mu$Ci/ml). The incubation was continued for 1 additional hr before harvesting the cells. $^3$H-labeled, thiolated, newly synthesized RNA was purified from equal quantities of total RNA by mercurated agarose affinity chromatography as described (1). After determination of radioactivity, these RNAs were subjected to RNA transfer blot analysis with IGF-I (Fig. 1*A*), albumin (Fig. 2*A*), and actin (Fig. 3*A*) probes. The same proportion of newly synthesized RNA, relative to the amount of total RNA from which it was purified, was loaded in each lane. This was accomplished by applying the same quantity of $^3$H radioactivity to each lane. Hybridization signals of the $^{32}$P-labeled probes hybridized to the newly synthesized RNAs were compared to the signals present in 10-$\mu$g aliquots of total, unfractionated RNA from the same cells electrophoresed on the same gels (Figs. 1–3 *B*).

In basal medium, no signal specific for IGF-I transcripts was apparent in the newly synthesized RNA (Fig. 1*A*), and little accumulation was seen in the unfractionated sample (Fig. 1*B*). In insulin-supplemented medium, a definite signal appeared in the newly synthesized RNA, and somewhat more had accumulated in the unfractionated sample relative to basal medium. In contrast, in the presence of GH or the combination of GH and insulin, a substantial signal was present both in the newly synthesized RNAs and in the unfractionated samples compared to that isolated from cells incubated in the presence of insulin alone. This implies that GH affects both transcription rates and total levels of accumulation of IGF-I transcripts.

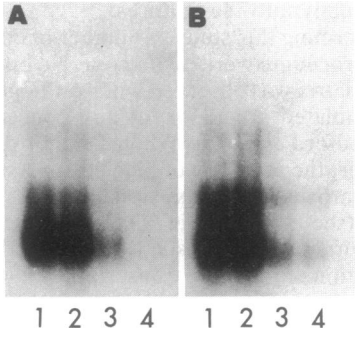


FIG. 1. Hybridization of IGF-I cDNA to thiol-labeled, newly synthesized RNA (*A*) and total, unfractionated RNA (*B*) prepared from hepatocyte cultures exposed to insulin and GH alone or in combination. Thiol labeling and RNA isolation were performed as described in the text. Lanes 1, GH plus insulin; lanes 2, GH; lanes 3, insulin; lanes 4, unsupplemented basal medium.

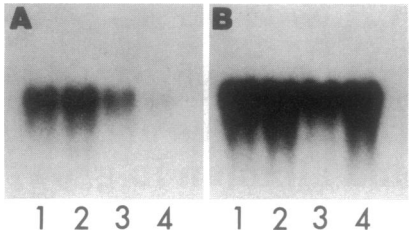


FIG. 2. Hybridization of albumin cDNA to thiol-labeled, newly synthesized RNA (*A*) and total, unfractionated RNA (*B*) prepared from hepatocyte cultures exposed to insulin and GH alone or in combination. Thiol labeling and RNA isolation were performed as described in the text. Lanes 1, GH plus insulin; lanes 2, GH; lanes 3, insulin; lanes 4, unsupplemented basal medium.

A similar analysis was carried out for the expression of albumin in cultured hepatocytes (Fig. 2). As for IGF-I, newly synthesized RNA isolated by mercurated agarose affinity chromatography from cells exposed to GH or GH and insulin contained elevated levels of albumin transcripts compared to that isolated from cells exposed to insulin alone, and the signal was barely detectable in RNA from cells exposed to basal medium (Fig. 2*A*). However, the total accumulated levels of albumin transcripts in the different hormonal conditions did not vary significantly (Fig. 2*B*). This pattern of accumulated transcript levels was markedly different from that of IGF-I (Fig. 1*B*).

A third pattern was apparent when the expression and accumulation of $\beta$-actin was analyzed (Fig. 3). Basal medium supported little or no transcription of actin, whereas addition of insulin, GH, or a combination of insulin and GH gradually increased the levels of actin transcripts in the newly synthesized RNA (Fig. 3*A*). Likewise, the total accumulated levels of actin transcripts were increased incrementally by addition of insulin, GH, and the combination of GH and insulin, respectively (Fig. 3*B*).

The data presented in Figs. 1–3 allow an estimation of the half-lives of IGF-I, actin, and albumin transcripts in the presence or absence of GH and insulin. In order to determine a transcript half-life, one needs to determine the value of the rate constant for degradation. Densitometric analysis of autoradiograms derived from Northern blots of thiolated, newly synthesized RNA allow this determination, provided

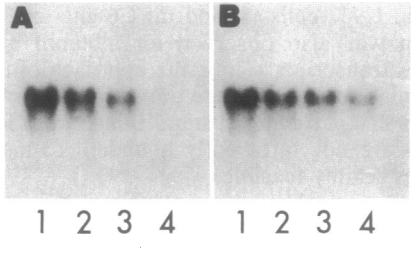


FIG. 3. Hybridization of actin cDNA to thiol-labeled, newly synthesized RNA (*A*) and total, unfractionated RNA (*B*) prepared from hepatocyte cultures exposed to insulin and GH alone or in combination. Thiol labeling and RNA isolation were performed as described in the text. Lanes 1, GH plus insulin; lanes 2, GH; lanes 3, insulin; lanes 4, unsupplemented basal medium.

that the thiol-labeled RNA is also double-labeled with $^3H$ and that RNA transcript levels are assumed to be in steady state during the thiol labeling period. As will be seen, the data allow this assumption to be tested. In brief, the principle is as follows: Northern blots are prepared from purified, newly synthesized RNA and from the unfractionated RNA from which the newly synthesized RNA was purified. The RNA samples are transferred to the same support and hybridized at the same time. The resulting autoradiogram is scanned, and the optical density for the signal of interest is determined for both RNA samples. By using the ratio of tritium radioactivity of the unfractionated and newly synthesized gel samples, the amount of hybridization signal optical density in the unfractionated RNA sample *that is due to newly synthesized transcripts* is calculated. Since the transcript levels are assumed to be steady state, this is equivalent to the amount of transcript that would have degraded over the labeling time period. Thus, one can calculate the fraction of transcripts left after the labeling period, and from this, the degradation rate constant is obtained. The assumptions and calculation will now be described in more detail.

The estimation requires three main assumptions: (*i*) Transcript levels are in steady state (i.e., the rate of transcript synthesis is equal to the rate of transcript degradation). (*ii*) Transcript degradation follows first-order kinetics. (*iii*) Little degradation of newly synthesized transcripts occurs during the time that the tritium and thiol labels are present.

First, it is necessary to calculate how much of the autoradiographic signal for a particular transcript in total, unfractionated RNA derived from hepatocytes exposed to a given hormone treatment is due to nascent, thiolated RNA synthesized during the 1-hr labeling period. For this purpose, the lanes in the autoradiogram are subjected to densitometric scanning in pairs. The pairs consist of a lane corresponding to total, unfractionated RNA derived from cells exposed to a particular hormone treatment and the lane corresponding to newly synthesized RNA purified from that sample. For example, Fig. 1*A*, lane 1 and Fig. 1*B*, lane 1 constitute such a pair. From the densitometric data and radioactivity measurements for the newly synthesized lane (Fig. 1*A*, lane 1 in the example), one obtains a defined value of optical density units per *unit sample* for each transcript. To define the unit sample, [$^3H$]uridine was included with 4-thiouridine during the labeling period. Thereby, the nascent thiolated RNA was also labeled with [$^3H$]uridine. The unit samples were defined by the measured radioactivity (cpm) applied to each lane. The $^3H$ label does not contribute to the signal of the $^{32}P$-labeled probe on the autoradiogram. The units of the defined optical density value per unit sample are thus those of a rate: optical density units·cpm$^{-1}$·hr$^{-1}$. This number is then multiplied by the $^3H$ radioactivity (cpm/hr) present in the other lane of the pair (Fig. 1*B*, lane 1 in the example)—i.e., the RNA from which the newly synthesized RNA was purified. The resulting number thus represents that portion of optical density in the autoradiographic signal from unfractionated RNA that is derived from hybridization to transcripts synthesized during the 1-hr labeling period ($OD_{ns\ in\ total}$). The lane corresponding to the unfractionated RNA (Fig. 1*B*, lane 1 in the example) is scanned to yield a value for total optical density for a particular transcript ($OD_{total}$), in which of course $OD_{ns\ in\ total}$ is included. The assumptions of steady state (i.e., that $OD_{ns\ in\ total}$ also equals the amount of transcript that degraded during the 1-hour labeling period) and first-order kinetics are invoked, and the relation

$$\frac{OD_{total} - OD_{ns\ in\ total}}{OD_{total}} = e^{-kt} \qquad [1]$$

is used to calculate the first-order rate constant $k$ for degradation, where $t$ is the labeling time (in this case 1 hr) and $e$ is the base for natural logarithms. Values for half-life are obtained from $k$.

The data may be analyzed further in order to assess the validity of the assumption of steady state on which the determination of half-life depends. The following analysis follows that given by Rodgers *et al.* (9). At steady state, the concentration $C_{ss}$ of a given transcript is determined by the relation

$$C_{ss} = k_s/k_d, \qquad [2]$$

where $k_s$ and $k_d$ are the (first-order) rate constants for synthesis and degradation, respectively. $C_{ss}$ can also be expressed as

$$C_{ss} = \frac{k_s t_{1/2}}{\ln 2} \qquad [3]$$

where $t_{1/2}$ is the transcript half-life. In our case, $C_{ss}$ is represented by the transcript levels in unfractionated RNA, and $k_s$ is represented by the transcript levels in newly synthesized RNA, or rather by the optical density per unit sample as defined previously, which is a rate constant (optical density units·cpm$^{-1}$·hr$^{-1}$). Thus, if the values for transcript levels and half-lives are normalized to those of one hormonal condition (e.g., insulin), for which all values have been set to unity, one would expect that the product of the normalized values for newly synthesized transcripts and half-life would be equal to the normalized value for total accumulation, if the assumption of steady state is valid. The choice of insulin incubation as a basis for normalization (i.e., closest approximation to steady state) was prompted by the fact that these cells had been incubated in insulin-containing medium for 48 hr prior to thiouridine addition and by the observation that under these culture conditions total RNA reaches constant specific activity in about 24 hr (data not shown).

The results of this kind of analysis, for two independent experiments on hepatocytes obtained from two different rats, are presented in Table 1. As can be seen, multiplying the relative rate of synthesis by the relative half-life of one of the three transcripts, calculated from measurements made in one incubation condition, yields a number (estimated relative total accumulation) that may be compared to the measured relative total accumulation for that transcript. We estimated the "goodness of fit" by using the following argument. Inspection of Eq. 3 indicates that the value of $C_{ss}$ will change by a minimum of 25% if the values of $k_s$ and $t_{1/2}$ deviate by 50% (independent of sign) from their steady-state values; this can be seen by expansion of the expression $(k_s \pm 0.5\ k_s)(t_{1/2} \pm 0.5\ t_{1/2})$. We considered data sets in which the values of the estimated relative total accumulation differed from the measured relative total accumulation by <25% to be consistent with a steady-state hypothesis. These sets are indicated by boldface italic type in Table 1. Numerical values for transcript half-lives for these sets are given in Table 2, together with a set of values for actin transcripts derived from actinomycin D experiments for comparison.

To establish whether 4-thiouridine itself altered the stability of RNA during the labeling period, the following experiment was performed. Rat C6 glial cells were incubated for 1 hr with [$^3H$]uridine at 15 $\mu$Ci/ml in the presence or absence of 100 $\mu$M 4-thiouridine. At this time the cells were washed, and the medium was replaced with fresh chase medium lacking 4-thiouridine and containing 1 mM unlabeled uridine. Cultures were harvested at time points up to 48 hr after addition of the chase medium, RNA was isolated, and

Table 1. Relative transcription rates, half-lives, and accumulated levels of IGF-I, actin, and albumin transcripts in cells exposed to GH and insulin for two experiments (1 and 2)

| Medium supplement | IGF-I | | | | | | Albumin | | | | | | Actin | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | RR | | $RT_{1/2}$ | | RTA | | RR | | $RT_{1/2}$ | | RTA | | RR | | $RT_{1/2}$ | | RTA | |
| | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| GH + insulin | ***3.0*** | 4.2 | ***0.7*** | 0.85 | ***2.3*** | 5.0 | 4.0 | 1.9 | 0.36 | 0.68 | 0.8 | 1.9 | 3.3 | 1.3 | 0.9 | * | 1.8 | 1.8 |
| GH | ***3.1*** | ***3.5*** | ***0.7*** | ***0.9*** | ***2.1*** | ***3.5*** | ***3.7*** | ***2.6*** | ***0.4*** | ***0.6*** | ***1.2*** | ***1.7*** | ***2.9*** | 1.3 | ***0.6*** | * | ***1.4*** | 1.2 |
| Insulin | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | * | 1 | 1 |
| None | — | — | — | — | — | — | 0.9 | ***0.84*** | 2.0 | ***2.7*** | 0.8 | ***1.9*** | ***0.7*** | 0.27 | ***0.5*** | * | ***0.45*** | 0.6 |

The calculations were based on densitometric scanning of autoradiograms from two separate experiments (1 and 2), both of which are shown. Transcription rates (i.e., optical density·$cpm^{-1}$·$hr^{-1}$ for newly synthesized transcripts) and half-lives (in hr) were calculated as described in the text. Accumulated levels are equivalent to $OD_{total}$ as described in the text. All values were normalized to those calculated for insulin-supplemented medium. RR, transcription rate relative to insulin-supplemented medium; $RT_{1/2}$, transcript half-life relative to insulin-supplemented medium; RTA, total accumulated transcript levels relative to insulin-supplemented medium. The sets of values indicated by boldface italic type meet the criterion of estimated relative total accumulation differing from measured total accumulation by <25% as described in the text. —, Not determined.

*These half-lives were deemed not valid since the value for insulin-supplemented medium was calculated to be less than the labeling time for 4-thiouridine.

specific activity of total RNA was determined. The pattern of change in total RNA specific activity with time was virtually identical in the cells incubated with 4-thiouridine as compared with controls. At 48 hr after initiation of the chase, the specific activity of RNA pulsed with 4-thiouridine and [$^3$H]-uridine was 82% of the specific activity at 24 hr. For cells pulsed with [$^3$H]uridine only without thiouridine, the specific activity of the RNA 48 hr after initiation of the chase was 84% of that at 24 hr. These results indicate that 4-thiouridine itself has no apparent effect on RNA stability.

## DISCUSSION

Our data indicate that GH stimulates the transcription of IGF-I, actin, and albumin genes. As indicated in Tables 1 and 2, the data also suggest that GH may act to destabilize these transcripts, particularly albumin. In our culture conditions, the rates of degradation and transcription of albumin appear to be controlled coordinately to maintain roughly constant levels of albumin mRNA. There are several other reports of constant albumin transcript levels maintained in culture in the context of widely varying rates of transcription, as measured by "run-on" assays (10, 11).

The analysis of thiolated, newly synthesized RNA transcripts is a useful adjunct to other approaches used to measure transcription rates and transcript stabilities. RNA nascent-chain elongation by isolated nuclei *in vitro* (run-on assay) is in widespread use as a way to measure instantaneous rates of transcription, and in systems where it has been tested it appears to faithfully reflect *in vivo* transcription rates (12). Its main disadvantages are that large numbers of nuclei and substantial amounts of radioactive RNA precursors are required if low rates of synthesis are to be detected; cells must be disrupted; and protocols are sometimes not easily transferable from one system to another, due to cell-specific differences in nuclear fragility or other factors. Moreover, since neither chain reinitiation nor chain termination occurs, run-on experiments do not represent normal transcription events and could lead to artifacts. By contrast, both plant and animal cells can be readily labeled in culture with 4-thiouridine by using fundamentally identical protocols (5, 13). For detection of low rates of synthesis, the limitations are the same as for detection of rare transcripts in conventional blotting procedures (i.e., the amount of available RNA and the *in vitro*-labeled probe specific activity). In the case of rare transcripts, a nuclease protection assay could be employed to detect thiolated nascent RNA. It should be pointed out, however, that in contrast to the run-on assay, blot analysis of newly synthesized RNA detects processed transcripts.

Table 2. Half-lives of IGF-I, albumin, and actin transcripts

| Medium supplement | IGF-I | | Albumin | | Actin | |
|---|---|---|---|---|---|---|
| | Exp. 1 | Exp. 2 | Exp. 1 | Exp. 2 | Exp. 1 | Exp. 3 |
| GH + insulin | 3.7 | | | | | 5.5 |
| GH | 3.5 | 2.9 | 12 | 9.6 | 2.6 | 4.6 |
| Insulin | 5.3 | 3.1 | 30 | 17 | 4.2 | 7.7 |
| None | — | — | | 46 | 2.4 | 4.9 |

Half-lives (in hr) of IGF-I, albumin, and actin transcripts from the data sets (indicated by boldface italic type in Table 1) that met the criterion of estimated total accumulation differing from measured total accumulation by <25% as described in the text are given. For comparison, values for actin transcripts are given that were derived from measurements of transcript decay in the presence of actinomycin D (Exp. 3). For this experiment, hepatocytes were incubated for 24 hr in the same four types of media (basal, with insulin, with GH, and with GH and insulin) as used for the thiolated RNA experiments. At this time, the medium was replaced with medium supplemented identically and containing in addition actinomycin D at 10 μg/ml, which suppressed [$^3$H]uridine incorporation by 98%. Cultures were harvested at the time of actinomycin D addition and at four time points thereafter, and RNA was isolated and subjected to Northern analysis with a β-actin cDNA as probe. The resulting autoradiograms were scanned, and half-lives were computed from a semilogarithmic plot of β-actin signal intensity versus time.

As a way to measure transcript half-life, the experimental approach presented here offers significant advantages over methods that employ transcriptional inhibitors or pulse–chase kinetics. Actinomycin D has been reported to both stabilize (14, 15) and destabilize (16) RNA. Our results indicate that 4-thiouridine has no apparent effect on RNA stability (cf. *Results*). The use of pulse–chase protocols is limited to relatively abundant transcripts. Although the concept used here is valid only in steady-state conditions, the data obtained allow an internal check of this assumption, as illustrated in Table 1. Moreover, in principle the approach described here could be used to measure very long-lived transcripts, which cannot be done with inhibitors of transcription, by increasing the time cells are incubated with 4-thiouridine.

Although the relative values for actin transcript half-lives in different hormone environments as measured by actinomycin D treatment were nearly the same as those determined by analysis of newly synthesized RNA (Table 2), the actual values determined by actinomycin D were about twice as long. This could be due to a stabilizing effect of actinomycin D as found by others (14, 15). Guyette *et al.* (2) found that half-lives for casein mRNA in cultured cells obtained by pulse–chase methods in glucosamine-treated cells were 25% of those measured by approach-to-steady-state methods and attributed this effect to toxicity of glucosamine and/or high concentrations of

unlabeled nucleotides. It is important, however, that the trends they observed (i.e., stabilization of casein mRNA by prolactin) were independent of methodology. We found that 100 $\mu$M 4-thiouridine in the culture medium decreased [$^3$H]-uridine incorporation by 50%, probably by competition since 100 $\mu$M unlabeled uridine suppressed [$^3$H]uridine incorporation by >95% (data not shown).

The values we obtained for albumin transcript half-life in hepatocyte cultures treated with insulin alone (17 and 30 hr) are similar to that found *in vivo* for diabetic rats or diabetic rats treated with insulin (22 hr; ref. 17). It is known that diabetic rats are deficient in GH secretion (18) and that hepatocytes' response to GH may be impaired even in insulin-treated diabetic rats (19). These effects could account for the shorter half-life for albumin transcripts measured in the presence of GH or the combination of GH and insulin (11–12 hr). The stimulation of albumin transcription by GH (Fig. 3 and Table 1) is consistent with the results of Idzerda *et al.* (20), who observed elevated levels of albumin and transferrin transcripts in mice made transgenic for the human or bovine GH gene under the control of the transferrin promoter.

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