Regulation by Thyrotropin-releasing Hormone (TRH) of TRH Receptor mRNA Degradation in Rat Pituitary GH3 Cells*

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In rat pituitary GH3 cells, thyrotropin-releasing hormone (TRH) down-regulates TRH receptor (TRH-R) mRNA (Fujimoto, J., Straub, R. E., and Gershengorn, M. C. (1991) Mol. Endocrinol. 5, 1527–1532), at least in part, by stimulating its degradation (Fujimoto, J., Narayanan, C. S., Benjamin, J. E., Heinflink, M., and Gershengorn, M. C. (1992) Endocrinology 130, 1879–1884). Here we show that TRH regulates RNase activity in GH3 cells and that specific mRNA sequences are needed for in vivo regulation of TRH-R mRNA by TRH. TRH affected RNase activity in a biphasic manner with rapid stimulation (by 10 min) followed by a decrease to a rate slower than in control lysates within 6 h. This time course paralleled the effects of TRH on degradation of TRH-R mRNA in vivo. The regulated RNase activity was in a polysome-free fraction of the lysates and was not specific for TRH-R RNA. A truncated form of TRH-R RNA that was missing the entire 3′-untranslated region (TRHR-R5) was more stable than full-length TRH-R RNA (TRHR-WT). In contrast to TRHR-WT mRNA, TRHR-R5 mRNA and TRHR-D9 mRNA, which was missing the 143 nucleotides 5′ of the poly(A) tail, were not down-regulated by TRH in stably transfected GH3 cells as their rates of degradation were not increased.

These data show that TRH regulates RNase activity in GH3 cells, that the 3′-untranslated region bestows decreased stability on TRH-R mRNA and that the 3′ end of the mRNA is necessary for regulation by TRH of TRH-R mRNA degradation. We present an hypothesis that explains specific regulation of TRH-R mRNA degradation by TRH in GH3 pituitary cells.

Regulation of mRNA degradation is an important process in controlling the expression of a number of proteins in different mammalian cells, but the mechanisms involved in this regulation have been delineated in only a limited number of instances (1–3). It appears there are both cis-acting elements, such as specific nucleotide sequences or secondary structures of mRNAs, and trans-acting factors, such as RNA-binding proteins and RNases, that are involved in regulated turnover of mRNAs. The rate at which a specific mRNA is degraded depends on the activity(ies) of the responsible RNase(s) and the stability (or susceptibility to degradation) of the mRNA.

Regulation of the activity of RNases in mammalian cells is poorly understood (4, 5). The RNase that degrades H4 histone mRNA is perhaps the best characterized of these RNases and appears to be a 3′ to 5′ exonuclease (6, 7). Its specificity, however, has not been well-defined and it is possible that it degrades other mRNAs in addition to that encoding histones. As the number of RNases in mammalian cells appears limited, it is likely that the specificity of regulated mRNA degradation is conferred by other factors that influence mRNA stability. RNA-binding proteins (8, 9) and specific nucleotide sequences and secondary structures in mRNAs (10–12) have been shown to be involved in regulating the specific degradation of mRNAs. In some cases in which the rate of degradation of a mRNA is increased, a regulatory protein has been shown to bind to a specific mRNA domain leading to a decrease in its stability.

Important insights into the mechanism of regulation of mRNA degradation may be obtained in studies in intact, living cells, and it is within this complex system that a complete mechanism must be defined. It is useful, however, to begin to study this process in a simpler model in which mRNA turnover can be isolated from transcription. By separating cytoplasmic processes from those occurring in the nucleus, a cell-free system can be used to characterize and isolate trans-acting factors that are involved in mRNA degradation. A number of studies using in vitro systems to delineate the factors involved in regulated mRNA degradation have been reported (13–16). In many of the reported studies, turnover of endogenous mRNAs was followed and important roles for polysome-associated factors (7, 17) and cytoplasmic factors (6, 14, 18) have been described. In some reports, exogenous RNAs were used to identify trans-acting cytoplasmic factors (19, 20).

The level of expression of cell-surface receptors in mammalian cells, for example, the transferrin receptor (9) and G protein- (guanine nucleotide-binding regulatory protein) coupled receptors, such as the β2-adrenergic (21, 22) and α2-adrenergic (23) receptors, are regulated in part by modulation of mRNA degradation. In rat pituitary GH3 cells and related cell lines, the number of receptors for TRH, which couples

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1The abbreviations used are: TRH, thyrotropin-releasing hormone; TRH-R, TRH receptor; TRHR-WT, full-length (wild type) mouse pituitary TRH-R; MeTRH, [N6-Me-His]TRH; Me, methyl; 3′-UTR, 3′-untranslated region; TRHR-D9, mouse pituitary TRH-R cDNA truncated at nucleotide 3356; TRHR-R5, mouse pituitary TRH-R cDNA missing the 3′-UTR; TRH-R, TRH response element; NEO, neomycin resistance gene; EGTA, [ethylenebis(oxy-ethylenenitri10)tetraacetic acid; MOPS, 4-morpholinepropanesulfonic acid.
via a G protein to activate inositol lipid hydrolysis (24, 25), is down-regulated by TRH (26, 27). TRH also causes the level of TRH-R mRNA to be down-regulated (28, 29). In a previous study (30), we used GH3 cells stably transfected with mouse pituitary THR-WT cDNA (31) to study regulation of TRH-R mRNA degradation. These transfectedants, therefore, allowed analysis of the degradation of TRH-R mRNA in an appropriate pituitary cell type in which regulation of transcription, which was driven by a viral promoter, was less important. This approach was selected, moreover, so that mutant cDNAs could be studied to gain insight into the structural features of TRH-R mRNA that may affect its turnover.

Here we study two aspects of the mechanism of regulated degradation of TRH-R mRNA by TRH. To determine the role of trans-acting regulatory factors, we have established a cell-free system derived from GH3 cells. Using this in vitro system, we show that TRH regulates a RNase activity that is found in a supernatant fraction of GH3 cell lysates. We also study the role of the 3'-UTR in TRH-regulated mRNA degradation in GH3 cells stably transfected with 'TRH-R cDNA, and show that the 3'-UTR bestows a more rapid rate of degradation on the mRNA and its 3'-end confers regulation by TRH.

**EXPERIMENTAL PROCEDURES**

**Materials**—[3H]MeTRH and [32P]UTP were purchased from Du Pont-New England Nuclear and myo-[3H]inositol was from Amer sham Corp. TRH and MeTRH were from Sigma. The expression vector pcDNA I/NEO was from Invitrogen. Geneticin (G418), Dulbecco's modified Eagle's medium, phosphate-buffered saline, and fetal calf and horse sera were from GIBCO. Nu-Serum and oligo(dT)-cellulose were from Collaborative Research. Restriction endonucleases were from Boehringer Mannheim and New England Biolabs. Mouse pituitary AtT-20/D16-F2 cells were from American Type Culture Collection. Cell Culture and Transfection—GH3 cells and GH1 cells stably transfected with mouse TRH-R cDNAs were grown in Dulbecco's modified Eagle's medium supplemented with 2.5% fetal calf serum and 15% horse serum as described (29). Cells were incubated in growth media at 37 °C and exposed to TRH at the doses and for the times indicated. GH3 cells, which were used because they had a low number of endogenous TRH-Rs, were transfected using DEAE-dextran and stable transfectedants were selected with G418 as described (30). The plasmid containing TRHR-WT cDNA was called pcNeoTMRHR in Fujiomoto et al. (28). It will be referred to as pmTRHR-WT here. GH3 cells stably transfected with pmTRHR-WT will be referred to as GHTMTRHR-WT cells, with pmTRHR-R5 as GHTMTRHR-R5 cells and with pmTRHR-D9 as GHTMTRHR-D9 cells. Mouse pituitary AtT-20/D16-F2 and mouse pituitary αT3-1 cells were grown in Dulbecco's modified Eagle's medium supplemented with 5% NuSerum.

**Receptor Binding Studies**—Binding to intact pituitary cells was performed under equilibrium conditions using [3H]MeTRH, a high affinity agonist for the TRH-R, as described (27). Binding data were analyzed using the LIGAND program (32).

**Inositol Phosphate Production**—Stimulation by TRH of inositol phosphate production in pituitary cells labeled with myo-[3H]inositol to isotopic steady-state was performed as described (33).

**Preparation of Cell Lysates**—Cells (20–30 × 10^6) were harvested with EDTA, centrifuged at 180 × g for 10 min at 4 °C, and the pellet washed three times with phosphate-buffered saline at 4 °C and resuspended in 0.25 ml of buffer A, 10 mM Tris acetate, 40 mM Kacetic acid, 3 mM magnesium acetate, pH 7.5, 5% glycerol. After autoclaving, 2 mM dithiothreitol, 0.57 mM phenylmethylsulfonyl fluoride, 0.1 mM sodium vanadate, 50 mM sodium fluoride, 2 μg/ml leupeptin, and 1 μg/ml aprotinin were added. The cells were homogenized in a 5-ml capacity glass homogenizer with a Teflon pestle using 20 strokes. The homogeneate was centrifuged at 12000 × g for 10 min at 4 °C, and the supernatant (“lysate”) was used immediately or frozen. For some experiments, the lysate (0.2 ml) was separated into supernatant (S-100) and polysome-containing fractions by centrifugation at 130,000 × g for 90 min at 4 °C. The resulting pellet was suspended in 0.1 ml of buffer A.

**In Vitro Transcriptions**—The transcription mixture (0.015 ml) contained 40 nm Tris-HCl, pH 7.6, 10 mM MgCl2, 1 mM spermidine, 10 mM NaCl, 10 mM diethoethrol, 20 units of RNasin, 0.5 mM ATP, 0.5 mM GTP, 0.6 mM CTP, 0.5 mM dCTP, 10 mM P-5'-(7-methyl)-guanosine-P-5'-guanosine triphosphate, 2 μg of linearized DNA template and 50 units of T7 RNA polymerase. The mixture was incubated for 30 min at 37 °C, 25 units of T7 RNA polymerase were added, and the incubation continued for 30 min. The mixture was extracted with phenol-chloroform, precipitated with ethanol, and the pellet was washed with 70% ethanol and resuspended in 0.1 ml of diethylpyrocarbonate-treated water.

**In Vitro Degradation System**—The degradation reaction mixture contained 2 μg of lysate (except where indicated), 20 μg of carrier tRNA, 20,000–30,000 cpm (12–15 ng) of [32P]RNA in 0.0125 ml of buffer A. Incubations were performed under equilibrium conditions in buffer A. Conditions in buffer alone. The degrading activity in lysates varied depending on the conditions of the assay. The rate of degradation in GH3 cells derived from GH3 cell lysates from control cells (Fig. 1). The half-maximally effective dose of 3 and 10 nM TRH is similar to that for...
stimulation by TRH of TRH-R mRNA degradation in vivo in GH3 cells stably transfected with mouse TRH-R cDNA (30). In most experiments, the increased activity was most readily observed in incubations containing 2 or 3 μg of lysate protein (data not shown). With greater amounts of lysate, 4 μg or greater, the rate of degradation was too fast to be measured accurately. The rate of degradation of TRH-R RNA was increased, but the effect of TRH was maintained when incubations were performed in the absence of unlabeled tRNA (data not shown). In contrast, there was no effect of pancreatic RNase inhibitor on the enhanced TRH-R RNA degradation observed in lysates from stimulated versus control cells. There were no specific degradation intermediates that accumulated during the incubations. In contrast to lysates from GH3 cells, TRH did not increase TRH-R RNA degrading activities in lysates from other rodent pituitary cells (AtT-20/D16-F2 and αT3-1 cells), which do not express TRH-Rs (data not shown).

TRH-R RNA degrading activity in lysates from GH3 cells was dependent on divalent cations. Total degrading activity and the increased degrading activity in lysates from TRH-stimulated GH3 cells were inhibited by increasing doses of EDTA and EGTA (data not shown). Degrading activity was abolished with 15 mM EDTA or 5 mM EGTA. Activities similar to those in incubations without chelators were attained with Mg2+/EDTA and Ca2+/EGTA ratios of 0.53 and 0.4, respectively. Antisense RNA hybridized to TRH-R [32P] RNA protected the RNA from degradation indicating that the RNase is single strand-specific.

Fig. 2 shows the time course of the effect of TRH to regulate TRH-R mRNA degradation in vivo in stably transfected GH3 cells. TRH stimulated an increase in TRH-R mRNA degradation that was maximal after 120 min; in these experiments, t1/2 of TRH-R mRNA degradation was 2.4 h in control cells, and 1.6 and 1.2 h in cells stimulated by TRH for 30 and 120 min, respectively. After 360 min of TRH exposure, the rate of TRH-R mRNA degradation had decreased and was slower than in control cells; t1/2 was 6.5 h. Fig. 3 illustrates the time course of the effect of TRH on TRH-R RNA degrading activity measured in the cell-free system. The time course of the TRH effect was similar to that measured in vivo. Increased degrading activity was found after 10 min of TRH exposure; in some experiments, an increase in degrading activity was found after 5 min of incubation of GH3 cells with TRH (data not shown). TRH stimulation of degrading activity was maximal after 30 min (2.5-fold control), remained at a similar level after 90 min, and was decreased thereafter. Similar to the findings in vivo, the degrading activity after 360 min was less than in lysates from control cells.

To determine whether the increase in RNA degrading ac-

Fig. 1. Dose-dependent effect of stimulation by TRH of GH3 cells in vivo on TRH-R RNA degradation in vitro. GH3 cells were incubated with various doses of TRH, lysates were prepared, and incubated with TRH-R [32P]RNA for 0, 10, 30 or 60 min, and RNA was analyzed as described under “Experimental Procedures.” These data are from one of three similar experiments.

Fig. 2. Time course of the effect of TRH to regulate TRH-R mRNA degradation in GH3 cells stably transfected with mouse TRHR-WT cDNA (GHmTRHR-WT cells). GHmTRHR-WT cells were incubated with 100 nM TRH for 30 (♂), 120 (●), or 360 min (▲) or without TRH (0 min, ○). Actinomycin D (5 μg/ml) was then added and replicate dishes were harvested at 0, 45, 90, or 180 min. TRH-R mRNA was measured by RNase protection assay. Data are the mean ± S.E. from three experiments.

Fig. 3. Time course of the effect of TRH stimulation of GH3 cells in vivo on TRH-R RNA degradation by cell lysates in vitro. GH3 cells were incubated with 100 nM TRH for 10 (♀), 30 (□), 90 (●), 180 (♂), or 360 min (▲) or without TRH (0 min, ○). Lysates were prepared and degradation of TRH-R [32P]RNA was measured as described under “Experimental Procedures.” Panel A, gel autoradiograms. Panel B, densitometric analysis of autoradiograms. Data are from one of three similar experiments.
To begin to delineate the site of action of TRH, we fractionated the lysates from control and TRH-treated cells. Fig. 4 illustrates the degradation of TRH-R RNA in lysate fractions that contained polysomes and in the high speed supernatant from control and TRH-stimulated GH3 cells. There was little or no degradation of TRH-R RNA in the polysome-containing fraction. In contrast, degrading activity was present in the supernatants from control and TRH-treated cells, and there was increased degrading activity in supernatant fractions from cells stimulated in vivo by TRH. In fact, virtually all of the degrading activity in total lysates was found in the supernatant fractions.

Fig. 5 illustrates the nucleotide sequence of TRHR-WT cDNA; the sequence of the 5' most 1752 nucleotides had been previously reported (31). Both strands of the 3'-UTR were sequenced. The TRHR-WT cDNA contains 3498 nucleotides and begins at nucleotide 1. The sequence of the 5' most 1752 nucleotides had been previously reported (31).

**Fig. 5. Nucleotide sequence of mouse TRHR-WT cDNA.** TRHR-WT cDNA contains 3498 nucleotides with the 3'-UTR extending from base 1438 to 3498 (plus a poly(A) tail). There are five copies of the AUUUA pentamer, a sequence that confers decreased stability to the TRH-R mRNA. Second, computer analysis showed the potential for a stable stem-loop secondary structure from nucleotides 3363 to 3453. There are six copies of the AUUUA pentamer in the coding region also. These sequences may be responsible for the relatively short half-life of the TRH-R mRNA. Two mutant cDNAs were constructed. TRHR-R5, which would have -800 bases of plasmid sequence in their 3'-UTRs, was truncated at nucleotide 1365 and was missing the entire structure from nucleotides 3363 to 3453.

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the rate of degradation of TRHR-WT mRNA, TRH caused a small decrease in the rate of degradation of TRH-R mRNA in GHmTRHR-D9 cells (Fig. 7) and had no effect on mRNA degradation in GHmTRHR-R5 cells (data not shown).

To further characterize the effects of TRH on TRHR mRNA degradation and the role of the 3'-UTR, we studied degradation of in vitro transcribed WT, D9, and R5 TRHR-RNAs in the cell-free system. TRHR-WT RNA contains a poly(A) tail because there is a tract of at least 40 adenosine residues in the TRHR-WT-cDNA whereas TRHR-D9 and R5 RNAs do not have poly(A) tails because the poly(A) tract has been excised. In lysates from unstimulated GH3 cells, R5 TRHR-R mRNA was more stable than D9 and WT RNAs, which were degraded at similar rates (Fig. 8). The effects of lysates from GH3 cells that had been stimulated in vivo with a maximally effective dose of TRH for various times are illustrated in Fig. 8. As shown in Fig. 3, there was a biphasic effect of TRH on TRHR-WT RNA degrading activity. The time course of TRH regulation of RNA degrading activity was similar when TRHR-WT, D9, or R5 RNAs were used to assess activity, but the magnitude of the effects were different. The RNase activity measured with all three RNAs was increased after 10 min of TRH stimulation in vivo. Maximum rates of degradation were attained after 30 min of TRH stimulation. The degradation rates of both WT and D9 TRHR-RNAs in lysates from GH3 cells stimulated by TRH for 30 min were increased 6-fold whereas degradation of R5 RNA was stimulated 3-fold. After 360 min of stimulation by TRH in vivo, the degrading activity assessed using TRHR-WT, D9, or R5 RNAs was lower than in lysates from unstimulated cells. Thus, the effect of TRH to regulate RNase activity was biphasic with a rapid increase followed by a decrease to below control levels after 360 min. It appears that the 3'-UTR confers greater instability on the RNA in lysates from unstimulated and TRH-stimulated cells. In contrast to the different effects of TRH on degradation of TRHR-D9, TRHR-R5, and TRHR-WT mRNAs in vivo, all three RNAs were degraded in vitro more rapidly by lysates from GH3 cells stimulated by TRH for 10-50 min than by lysates from control cells.

## DISCUSSION

TRH causes the level of endogenous TRH-R mRNA to decrease in rat pituitary GH3 cells, at least in part, by stimulating the rate of its turnover (28, 29). This effect of TRH could have been caused by decreasing the stability of TRH-R mRNA (or increasing its accessibility to a RNase) or by increasing the activity(ies) of a RNase(s) that degrades TRH-R mRNA, or both. To determine whether TRH affects the activity of a RNase that degrades TRH-R RNA, we used a

### TABLE I

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<th>Cell line</th>
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<th>TRH-R binding*</th>
<th>Kd</th>
<th>Bmax</th>
<th>Emax</th>
<th>Maximum</th>
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<td>11 ± 2.4</td>
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<tr>
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<td></td>
<td>0.7 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td>2.5 ± 0.5</td>
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<tr>
<td>GHmTRHR-D9</td>
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<td>0.6 ± 0.1</td>
<td>2.3 ± 0.5</td>
<td>8.4 ± 1.9</td>
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*TRH-R binding was measured in intact cells and the maximum binding (Bmax) is presented per mg of cell protein.

TRH stimulation was performed for 30 min.

### Fig. 6. Effects of TRH on the levels of transcribed TRH-R mRNAs in GHmTRHR-WT, GHmTRHR-R5, and GHmTRHR-D9 cells.

Cells were incubated without TRH or with 100 nM TRH for the indicated times. Total cell RNA was extracted and the level of TRH-R mRNA was measured by RNase protection assay as described under "Experimental Procedures." Data represent mean ± S.D. of triplicate points from one of two similar experiments.
cell-free system in which the RNA degrading enzyme(s) was from lysates of TRH-stimulated and unstimulated GH3 cells. The input RNA was transcribed in vitro and, therefore, was not decorated with cellular binding proteins or associated in large ribonucleoprotein complexes as RNA likely is in vivo. We found that lysates derived from GH3 cells stimulated by TRH have regulated activity to degrade in vitro transcribed RNAs. This activity has characteristics that are similar to some previously described mammalian RNases that are involved in regulated degradation of other mRNAs (6, 15). The RNase activity from GH3 cells is dependent on divalent cations, is not inhibited by placental RNase inhibitor, is specific for single-stranded RNA, and appears to degrade RNAs progressively without forming any specific degradation intermediates. The latter observation is consistent with this enzyme being an exonuclease, most likely one that degrades RNA in the 3’ to 5’ direction, as has been shown for the RNase that degrades H4 histone mRNA (7). This is different from the RNase activity that is stimulated in Xenopus liver by estrogens that may be an endonuclease (38). Another feature of the cell-free system, which has been described in another regulated in vitro RNA degrading system (39), is that the regulated degrading activity is more readily observed when the amount of cell lysate, that is, total degrading activity, is increased in GH3 cells stimulated by TRH (41). We think the in vitro findings are important and that the lack of specificity found in the cell-free system can be explained by the complexity of the system that regulates mRNA turnover in vivo.
(2). In cells, mRNA degradation is influenced by cis-acting elements, such as specific mRNA sequences or secondary mRNA structures, and trans-acting factors, such as RNA-binding proteins and RNases. The rate of degradation of a specific mRNA is a complex function of all of these influences. We think, therefore, that in GH3 cells stimulated by TRH that some mRNAs, such as that for prolactin, are protected from increased degradation during TRH action by effects on other components of the RNA degrading system. For example, TRH may simultaneously increase the activity of a trans-acting regulatory factor that stabilizes prolactin mRNA. TRH stimulation of GH3 cells appears to lead to degradation of TRH-R mRNA by increasing mRNA stability and simultaneously affecting a specific factor(s) that allows for degradation of (or destabilizes) TRH-R mRNA. We found that the effect of TRH to down-regulate TRH-R mRNA in GH3 cells is dependent on a domain in the 3'-end of the TRH-R mRNA. In GH3 cells transfected with TRH-R cDNAs that lacked the 3'-end of the mRNA, TRH did not decrease TRH-R mRNA levels (Fig. 6) or increase their degradation (Fig. 7). In contrast, in the cell-free system, lysates from GH3 cells stimulated by TRH for less than 90 min degraded all in vitro transcribed TRH-R RNAs more rapidly than lysates from unstimulated (control) cells (Fig. 8). Hence, it is likely that there is (are) an additional factor(s) that interacts with the 3'-end of the TRH-R mRNA that confers specific regulation of TRH-R WT mRNA degradation in vivo (see below).

Regulated degradation of several mRNAs in mammalian cells has been shown to depend on specific mRNA sequences or secondary structures. Perhaps the most widely studied mechanism involves shortening of the poly(A) tail that appears to be the first step in stimulated degradation of some mRNAs (12). A different mechanism appears to account for rapid degradation of other mRNAs including those for c-myc, c-fos, and granulocYTE-macrophage colony-stimulating factor (42-44). These mRNAs contain AU-rich regions having AUUUA pentamers that when inserted into a usually stable mRNA can destabilize it (45). It may be that binding of a protein to AU-rich sequences in the 3'-UTR of these mRNAs causes changes in mRNA stability. A protein that binds specifically to these sequences has been reported (46); however, it has not yet been shown to influence mRNA stability directly (47). This mechanism may be involved in TRH-R mRNA regulation as the 3'-UTR of the TRH-R mRNA is AU-rich and contains a AUUUAAUUUA motif (Fig. 5). A variation on this model has been proposed to explain estrogen stimulation of the degradation of apolipoprotein II mRNA in frogs from chickens (48). In this system, AU-containing trinucleotides were found to direct endonucleolytic cleavage of this mRNA. These sequences, therefore, may be involved in stabilization-destabilization of a class(es) of mRNAs but do not likely mediate the specific turnover of a single mRNA species. An example of a specific mRNA sequence-structure that confers stability-instability on a single mRNA has been reported for transferrin receptor mRNA (9). Regulation of transferrin receptor mRNA appears to involve binding of a protein (49) to a specific sequence in a stem-loop structure in the 3'-UTR of the mRNA (10, 11). Binding of this iron response element-binding protein to transferrin receptor mRNA, which occurs in a low iron environment, stabilizes the mRNA. (This protein appears to bind to the 5'-UTR of ferritin mRNA also and decreases its translation (20).) This mechanism is specific because it involves binding of a regulatory protein to a specific mRNA sequence-structure. A mechanism analogous to this could account for the specific degradation of TRH-R mRNA in GH3 cells in the presence of increased nonspecific RNase degrading activity (see below).

We show that the 3'-UTR confers decreased stability on TRH-R mRNA. This was evident when the half-lives of the TRHR-WT and TRHR-R5 mRNAs were compared in transfected GH3 cells and was even more apparent when their degradation rates were compared in vitro (Fig. 8). As noted above, the 3'-UTR of TRH-R mRNA contains AU-rich regions with AUUUAA pentamers and these may be responsible for its relatively short half-life. Our finding of retention of a similar order of rates of degradation for the different TRH-R mRNAs in vivo to that in vitro in transfected GH3 cells (TRHR-WT similar to TRHR-D9, both faster than TRHR-R5) adds support to the idea that the cell-free system can be used as a model system in which to delineate the processes involved in mRNA degradation in vivo. These data show, furthermore, that stability is in part an intrinsic property of the mRNAs because in the in vitro system the transcribed RNAs are not likely to be associated with RNA binding proteins that would affect their stability in vivo.

Although there are a number of mechanisms that could account for our observations, we favor the following model that incorporates ideas developed from our findings in vitro and in vivo studies using GH3 cell lysates and in vivo studies in transfected GH3 cells. Our hypothesis is that regulated TRH-R mRNA degradation involves a cis-acting element within the mRNA and is mediated by two transient effects of TRH on trans-acting factors. One effect leads to destabilization of TRH-R mRNA and the other is to increase the activity of a RNase that degrades TRH-R mRNA. Because both of these effects are necessary TRH stimulation of TRH-R mRNA degradation is made specific and rapid. In our model, the TRH-R mRNA can be divided into several regions that affect its turnover (Fig. 9). The 5'-untranslated region and the coding region, comprising nucleotides 1-1437, is one domain that is relatively stable. Regions within the 3'-UTR confer relative instability, most likely because the 3'-UTR contains AU-rich domains and a AUUUAAUUUA motif. These elements in the 3'-UTR, which are not specific for TRH-R mRNA, determine the basal rate of degradation. Because these elements confer relatively rapid turnover under certain conditions these domains are important in allowing for rapid changes in TRH-R mRNA levels during TRH stimulation. Specificity to regulated degradation is conferred by a response element within the 143 nucleotides 5' of the poly(A) tail. This region of the TRH-R mRNA appears to contain a stable stem-loop structure between nucleotides 3363 and 3453. We think that this stem-loop structure is the cis-acting TRH-RE. The iron response element in the transferrin receptor mRNA appears to be in a stem-loop structure also (9). TRH stimulates a RNase that can degrade at least several RNAs in vitro and is, therefore, not specific. We propose that most mRNAs in vivo are not degraded more rapidly during TRH action because they do not contain a TRH-RE and, therefore, are not simultaneously destabilized. We suggest that a TRH-RE-binding protein mediates specific destabilization of TRH-R mRNA. We suggest, furthermore, that during TRH stimulation, this regulatory protein is phosphorylated, perhaps by protein kinase C, which is activated during TRH action (50). Phosphorylation of this protein might increase its affinity for the
TRH-RE causing it to bind to and destabilize TRH-R mRNA. Regulation of TRH-R mRNA stability would, therefore, be mediated by phosphorylation/dephosphorylation of a specific RNA binding regulatory protein. A similar mechanism has been proposed, although not proved, for changes in mRNA stability caused by an adenosine-uridine binding factor (46, 47, 51). It is of note, that phosphorylation-dephosphorylation has been found to regulate the binding of transcription regulatory proteins also (52). Lastly, our model does not include shortening of the poly(A) tail as an initial step in regulated TRHR-R mRNA degradation (12), but this could be part of the mechanism and may occur secondary to binding of the regulatory protein to the TRH-RE, which is situated 5' of the poly(A) tail.

In conclusion, we present data that provide initial insights into the complex events that mediate TRH regulation of TRH mRNA in GH3 pituitary cells. These data show that TRH regulation of TRH-R mRNA levels involves effects on the activity of a TRH-R mRNA-degrading RNase and on the stability of the mRNA. Future work will attempt to define the molecular details of TRH stimulation of RNase activity and of TRH-induced destabilization of TRH-R mRNA.

REFERENCES