Luteinizing hormone (LH) receptor mRNA is post-transcriptionally regulated. An ovarian cytosolic LH receptor mRNA-binding protein (LRBP) identified in our laboratory binds to a polypyrimidine-rich bipartite sequence in the coding region of LH receptor mRNA. The present studies show a role for LRBP in the regulation of LH receptor mRNA. We demonstrated that increased LH receptor mRNA degradation occurs during hormone-induced LH receptor down-regulation. Furthermore, increased degradation of LH receptor mRNA was seen when partially purified LRBP was included in an in vitro mRNA decay reaction. The LH receptor mRNA binding activity of LRBP measured by RNA electrophoretic mobility shift analysis showed an inverse relationship to LH receptor mRNA levels during different physiological states. These results suggest that LRBP is a physiological regulator of LH receptor mRNA expression in the ovary and provides a novel mechanism for the regulation of LH receptor expression in the ovary.

The expression of luteinizing hormone receptors (LHR)¹ on the rat ovarian granulosa cells and luteal cells is decreased by an endogenous preovulatory luteinizing hormone (LH) surge or by the administration of a pharmacological dose of human chorionic gonadotropin (hCG), a placental counterpart of LH (1–4). Studies from our laboratory have demonstrated that the decline in cell surface LHR number seen after hCG administration is paralleled by a specific loss of LHR mRNA (2, 3). The expression of luteinizing hormone receptors (LHR)¹ on the rat ovarian granulosa cells and luteal cells is decreased by an endogenous preovulatory luteinizing hormone (LH) surge or by the administration of a pharmacological dose of human chorionic gonadotropin (hCG), a placental counterpart of LH (1–4). The day of hCG injection is taken as day 0. LH receptor down-regulation was induced by the injection of 50 IU of pregnant mare serum gonadotropin followed by 25 IU of hCG 56 h later.

Animals and Tissues—Pseudopregnancy was induced in 21-day-old Sprague-Dawley female rats by a subcutaneous injection of 50 IU of pregnant mare serum gonadotropin followed by 25 IU of hCG 56 h later. The day of hCG injection is taken as day 0. LH receptor down-regulation was induced by the injection of 50 IU of hCG on the fifth day of pseudopregnancy. Ovaries were collected at the indicated times and were processed immediately.

Preparation of Polysomes—Ovaries from control (pseudopregnant) and hormone-treated (12-h post-hCG-injected) animals were homogenized in solution A (1 mM potassium acetate, 2 mM Mg(Ac)₂, 2 mM dithiothreitol, and 10 mM Tris acetate, pH 7.6) at 4 °C. After centrifugation for 10 min at 10,000 × g, the supernatants were layered over a macro-prep High Support column was obtained from Bio-Rad. RNase inhibitor mixture tablets, RNase T1, and Quik spin columns (G-50 Sephadex) for radiolabeled RNA purification were obtained from Roche Molecular Biochemicals. mMessage mMachine Kit was a product of Ambion (Austin, TX). RNasin was purchased from Promega (Madison, WI). Macro-prep High S Support column was obtained from Bio-Rad. Centriplus YM-10 and Centricon YM-10 microconcentrators were products of Millipore (Bedford, MA).

Methods—Pseudopregnancy was induced in 21-day-old Sprague-Dawley female rats by a subcutaneous injection of 50 IU of pregnant mare serum gonadotropin followed by 25 IU of hCG 56 h later. The day of hCG injection is taken as day 0. LH receptor down-regulation was induced by the injection of 50 IU of hCG on the fifth day of pseudopregnancy. Ovaries were collected at the indicated times and were processed immediately.

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In vitro mRNA Decay Assay—Half-life of LHR mRNA was determined by an in vitro decay reaction based on the protocol developed by Ross (7). An A₂₆₀ of 0.6—1.0 units of polysomes were mixed gently in 25 μl (final volume) of solution A (as described under polysome isolation) supplemented with 10 μM creatine phosphate, 0.04 mg/ml creatine kinase, 1 mM ATP, 0.2 mM GTP, 0.1 mM potassium acetate, 0.1 mM spermine, 0.8 mM Mg(Ac)₂, and plasmid RNase inhibitor (400 units/ml). The in vitro decay reactions were incubated at 16 °C for 0, 15, 30, 60, and 120 min. Where indicated, total RNA isolated from control pseudopregnant rat ovaries and partially purified LRBP were also included in the reaction. After incubation at intervals shown above, the reactions were stopped. RNA in the reaction tubes was extracted immediately using the acid guanidinium isothiocyanate method as described by Chomczynski and Sacchi (8), and LHR mRNA was detected by Northern blot analysis.

Materials and Methods

Chemicals—Pregnant mare serum gonadotropin was purchased from Calbiochem. Human chorionic gonadotropin was obtained from Sigma. [α-³²P]dTTP was purchased from ICN (Costa Mesa, CA), and [α-³²P]UTP was from PerkinElmer Life Sciences. EDTA-free protease inhibitor mixture tablets, RNase T1, and Quik spin columns (G-50 Sephadex) for radiolabeled RNA purification were obtained from Roche Molecular Biochemicals. mMessage mMachine Kit was a product of Ambion (Austin, TX). RNasin was purchased from Promega (Madison, WI). Macro-prep High S Support column was obtained from Bio-Rad. CargoNT YM-10 and Centricon YM-10 microconcentrators were products of Millipore (Bedford, MA).

Post-transcriptional Regulation of Luteinizing Hormone Receptor mRNA in the Ovary by a Novel mRNA-binding Protein

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"The abbreviations used are: LHR, luteinizing hormone receptors; LH, luteinizing hormone; hCG, human chorionic gonadotropin; LRBP, LH receptor mRNA-binding protein; PMSG, pregnant mare serum gonadotropin.

Life (4). Additional studies led to the identification of a 50-kDa LHR mRNA-binding protein designated as LRBP in rat and human ovarian cytosolic fractions. During hormone-induced down-regulation of the LHR, the LHR mRNA binding activity of LRBP was increased. LRBP specifically binds to the coding region of LHR mRNA with an apparent dissociation constant of 10⁻⁹ M (5, 6). These studies were carried out to determine the role of LRBP in LHR mRNA degradation in vitro as well as to establish a relationship between LHR mRNA expression and LRBP during ovarian development. Our results show that LHR mRNA expression inversely correlates with the LHR mRNA binding activity of LRBP during follicular maturation, ovulation, and luteinization. Furthermore, a partially purified LRBP causes accelerated decay of LHR mRNA in an in vitro reconstituted mRNA decay system.

In Vitro mRNA Decay Assay—Half-life of LHR mRNA was determined by an in vitro decay reaction based on the protocol developed by Ross (7). An A₂₆₀ of 0.6—1.0 units of polysomes were mixed gently in 25 μl (final volume) of solution A (as described under polysome isolation) supplemented with 10 μM creatine phosphate, 0.04 mg/ml creatine kinase, 1 mM ATP, 0.2 mM GTP, 0.1 mM potassium acetate, 0.1 mM spermine, 0.8 mM Mg(Ac)₂, and plasmid RNase inhibitor (400 units/ml). The in vitro decay reactions were incubated at 16 °C for 0, 15, 30, 60, and 120 min. Where indicated, total RNA isolated from control pseudopregnant rat ovaries and partially purified LRBP were also included in the reaction. After incubation at intervals shown above, the reactions were stopped. RNA in the reaction tubes was extracted immediately using the acid guanidinium isothiocyanate method as described by Chomczynski and Sacchi (8), and LHR mRNA was detected by Northern blot analysis.
by Northern blot analysis as described below. The 6.7-kb LHR mRNA (major transcript) was quantitated in densitometric units using NIH image 1.61 software. The half-life (t_{1/2}) of LHR mRNA was calculated by plotting the corresponding densitometric units of LHR mRNA versus incubation time.

**Northern Blot Analysis**—Total RNA was extracted using a previously described procedure (8). Ovaries were homogenized in a solution of guanidine isothiocyanate, acidified with 2 M sodium acetate, pH 4.0, and extracted with water-saturated phenol and chloroform-isomyl alcohol (49:1). RNA was precipitated at -20 °C using three volumes of ethanol and was quantitated by UV-absorbance spectroscopy. Northern blot hybridization analysis was performed essentially as described by Maniatis and colleagues (9). The LHR cDNA probe used for Northern blot hybridization has been described earlier (4). A 1.7-kb cDNA for human cytochrome P450scc was used as probe for Northern blot analysis (10). The intensity of LHR and P450scc mRNA was quantified in densitometric units and normalized to 18 S rRNA.

**Preparation of Full-length LHR cDNA**—The 2.1-kb LHR cDNA containing the full-length LHR coding region was ligated into pBluescript II between XbaI and BamHI sites and was used to generate full-length LHR mRNA. Radiolabeled RNA was prepared from linearized template using mMessage mMachine kit in the presence of 100 μCi of [32P]UTP. Following transcription, RNA was extracted with nuclease-free water-saturated phenol-chloroform-isomyl alcohol (50:49:1). Unincorporated nucleotides were removed from the labeled RNA using Quick spin columns (G-50-Sephadex). RNA was precipitated with equal volume of isopropyl alcohol at -20 °C. Precipitated RNA was then washed with 75% ethanol air-dried and was dissolved in nuclease-free water.

**RNA Gel Shift Analysis**—Unless otherwise indicated, RNA gel shift analysis was performed as described previously (6). Protein samples were incubated with 1 × 10^5 cpm of radiolabeled gel-purified RNA in homogenization buffer A described above in the presence of 5 μg of tRNA and 40 units of RNasin at 30 °C for 10 min. Unprotected radiolabeled RNA was then degraded by the addition of 2 units of RNase T1 at 37 °C for 30 min. Samples were then incubated with heparin at a final concentration of 5 mg/ml for 10 min on ice to decrease nonspecific binding. The RNA-protein complexes were resolved by 8% native polyacrylamide gel electrophoresis at 4 °C. The gel was then dried and exposed to Kodak X-Omat AR film and visualized by autoradiography. Radiolabeled bands were quantitated in densitometric units using NIH image 1.61 software.

**Statistical Analysis**—Each experiment was repeated at least three times, and the results presented represent a single experiment. Error bar represents the mean ± S.E. of three separate densitometric scans.

**RESULTS**

**In Vitro Decay of Endogenous and Exogenous LHR mRNA**—The decay of endogenous and exogenously provided LHR mRNA was examined in a cell-free system. The polysomes were isolated from day 5 pseudopregnant rat ovaries when LHR mRNA expression was abundant. The decay of endogenous LHR mRNA associated with polysomes was determined by incubating polysomes in an in vitro decay reactions as described under Materials and Methods. The incubations were carried out at 16 °C for 0, 15, 30, 60, and 120 min. At the end of this incubation period, total RNA was extracted from each fraction, and LHR mRNA was determined by Northern blot using a LHR cDNA probe. The results presented in Fig. 1A show all four LHR mRNA transcripts (6.7, 4.4, 2.6, and 1.8 kb) endogenously associated with the polysomes remaining at different time intervals. The loss of all four transcripts occurred concomitantly. The 6.7-kb transcript is the predominant LHR mRNA in rat ovary. Panel C represents the rate of degradation in densitometric units of the 6.7-kb transcript in panel A normalized to 18 S rRNA. The degradation of endogenous LHR mRNA occurred rapidly (t_{1/2} = 58 min), and by 120 min, there were minimal detectable levels of LHR mRNA remaining. Panel B shows 18 S rRNA determined by Northern blot hybridization for normalizing RNA loading. The level of 18 S rRNA was not altered during the 120-min incubation period. Because the degradation of endogenously associated LHR mRNA occurred rapidly, in subsequent experiments 15 μg of total RNA extracted from pseudopregnant rat ovaries were included to ensure that RNA was not limiting in the in vitro decay reaction.

Because our previous studies have shown that the degradation of LHR mRNA occurs more rapidly during hormone-induced down-regulation (2–4), attempts were made to examine whether the decay of exogenous LHR mRNA by polysomes from LHR-down-regulated rat ovaries occurred at a rate faster than the degradation of endogenous LHR mRNA by polysomes isolated from control ovaries. For this study, polysomes were isolated from both LHR-down-regulated and control ovaries. Reactions containing polysomes from control and LHR-down-regulated ovaries were then separately incubated with exogenously added total RNA for up to 120 min, and the reactions were terminated at different time intervals as shown in Fig. 2. RNA was extracted, and Northern blot hybridization was per-
formed using LHR cDNA probe. Panel A shows the 6.7-kb LHR mRNA transcript remaining at different time intervals in each group. Panel C shows 18 S rRNA in each fraction. Panel D depicts the densitometric scans of the 6.7-kb transcript (major transcript in rat ovary) in panel A normalized for 18 S rRNA. The results show that the decay of exogenously added LHR mRNA occurred with a \( t_{1/2} \) of 46 min when incubated in decay reactions using polysomes isolated from control ovaries. The decay of exogenous LHR mRNA proceeded at a markedly faster rate with a \( t_{1/2} \) of \( \approx 10 \) min in the decay reactions with polysomes isolated from hormone-induced LHR-down-regulated rat ovaries. To determine whether this rapid degradation of LHR mRNA by polysomes from LHR-down-regulated rat ovaries was selective for LHR mRNA, we examined the degradation of cholesterol side-chain cleavage enzyme cytochrome P450sccc mRNA by stripping the same blot and rehybridizing with \(^{32}\text{P}\)cytochrome P450sccc cDNA probe as described under “Materials and Methods.” Fig. 2B shows the cytochrome P450sccc mRNA remaining at different time intervals of the in vitro degradation reaction in each group. Panel D depicts the rate of degradation in densitometric units of cytochrome P450sccc in panel B normalized to 18 S rRNA (panel C) for the difference, if any, in RNA loading. There was no discernible difference in the rate of degradation of cytochrome P450sccc between control and LHR-down-regulated groups. This finding indicates that the LHR mRNA degradation by polysomes from LH receptor-down-regulated rat ovaries is selective for LHR mRNA.

LHR mRNA Degradation in Vitro by Partially Purified LRBP—To determine whether the partially purified LHR mRNA-binding protein LRBP induces accelerated decay of LHR mRNA in vitro, polysomes isolated from control ovaries were incubated with exogenously added LHR mRNA and partially purified LRBP. The assays were carried out as described under “Materials and Methods.” Aliquots of decay reactions were incubated for 0, 15, 30, 60, and 120 min at 16 °C, and LHR mRNA content was detected by Northern hybridization analysis (Fig. 3A). An addition of partially purified LRBP (70 μg) increased the degradation of LHR mRNA when compared with incubations with no LRBP. The decay of LHR mRNA was so rapid that there was an undetectable level of the 6.7-kb transcript at 120 min. Half-lives of LHR mRNA were calculated from the densitometric scans of the 6.7-kb transcript in decay reactions performed in the presence of LRBP or with equal volume of buffer as shown in Fig. 3B. The \( t_{1/2} \) of exogenously added LHR mRNA was reduced to 26 min in the presence of partially purified LRBP. The \( t_{1/2} \) of LHR mRNA in control reactions was 38 min. To address whether the degradation of LHR mRNA is dependent on LRBP concentration, in vitro decay reactions with control polysomes and exogenously added LHR mRNA were performed with different concentrations (9.6, 19.2, 38.5, and 77 μg) of partially purified LRBP at 16 °C for 60 min. As shown in Fig. 4, an increased degradation of LHR mRNA was observed by increasing the LRBP concentration in the decay reactions when compared with that seen in its absence. These data indicate that LRBP, which binds to the...
Methods. Aliquots of the decay reactions were incubated for 60 min in the absence of added protein (C) and in the presence of increasing concentrations of LRBP (9.6–77 μg). LHR mRNA content was assayed by Northern blot analysis, quantitated by densitometric scanning, and normalized for 18S rRNA.

Changes in LHR mRNA Binding Activity of LRBP during PMSG and hCG-induced Regulation of LHR mRNA—Experiments were then conducted to determine whether LHR mRNA binding activity of the LRBP present in the ovarian S-100 fractions bears any relationship to the steady-state levels of LHR mRNA. First, the changes in LHR mRNA binding activity of the LRBP and LHR mRNA expression were determined after hormone treatment. 21-day-old rats were treated with 50 IU of PMSG to induce follicular maturation, and ovaries were collected 56 h later. LHR mRNA expression was examined by Northern blot analysis and the LHR mRNA binding activity of LRBP by RNA electrophoretic mobility shift analysis using the S-100 fractions isolated from the ovaries. The results (Fig. 5, A, C, and D) show that expression of LHR mRNA increased slightly 56 h after PMSG injection, but LRBP activity in the S-100 fraction showed a decline at this time interval compared with the pretreatment level. The PMSG-treated animals were then treated with 25 IU of hCG to induce ovulation and corpus luteum formation. Ovaries were collected at 6, 12, 24, 48, and 72 h after hCG injection to determine LHR mRNA expression and the LHR mRNA binding activity of LRBP. Upon treatment with hCG, as expected, the levels of LHR mRNA started to decline by 6 h and reached the lowest level by 12 h. The LHR mRNA expression started to increase by 24 h and further increased by 72 h (Fig. 5, A and D) following hCG treatment. RNA electrophoretic mobility shift analysis was performed in S-100 fractions prepared from ovaries collected at the same time intervals as those used for determining LHR mRNA levels. The intensities of the ribonucleoprotein complexes formed (Fig. 5C) were quantitated by densitometric scanning (Fig. 5D). The results show that following hCG injection, the LHR mRNA binding activity of LRBP showed an increase at 6 and 12 h. The LHR mRNA binding activity of LRBP in the S-100 fractions started to decrease by 24 h after hCG injection. This finding is in sharp contrast to the pattern of LHR mRNA expression seen at different time intervals following hCG treatment. The results show that in all instances the expression of LHR mRNA inversely correlated with the LHR mRNA binding activity of LRBP.

**LHR mRNA Expression and LHR mRNA Binding Activity of LRBP during Pseudopregnancy**—Because the corpus luteum has a defined life span and the expression of LHR mRNA shows marked changes during this period, both LHR mRNA levels and the LHR mRNA binding activity of LRBP were assayed to examine whether a correlation exists between the two.

Pseudopregnancy was induced by a subcutaneous injection of 50 IU of PMSG to 21-day-old rats followed by treatment with 25 IU of hCG 56 h later. The day of hCG injection was taken as day 0 of pseudopregnancy. Ovaries were collected at 2, 4, 6, 8, 10, 12, and 14 days of pseudopregnancy for LHR mRNA expression and RNA electrophoretic mobility shift studies as described in earlier experiments and under “Materials and Methods.” Fig. 6A represents the Northern blot showing the expression of LHR mRNA at different days of pseudopregnancy. Following a steady increase in LHR mRNA expression culminating in maximum expression on day 8, a sharp decline was seen after day 8 of pseudopregnancy extending to day 14. The LHR mRNA binding activity of LRBP in ovarian S-100 fractions at the same time interval is shown in Fig. 6C, and the intensities of these ribonucleoprotein complexes were quantitated densitometrically (Fig. 6D). The results show that the LHR mRNA binding activity of LRBP increased from days 4–10 of pseudopregnancy. The maximum binding activity of
LHR mRNA decay reactions performed with polysomes isolated from control and hCG-induced LHR-down-regulated ovaries permit reconstitution of LHR mRNA-degradative activity in a cell-free system. The accelerated degradation of LHR mRNA in the presence of partially purified LRBP in vitro presented in this report indicates that LRBP acts as a trans-acting factor to induce the decay of LHR mRNA.

Earlier studies from our laboratory have shown that the LHR mRNA binding activity of LRBP in the ovarian S-100 fraction increases during hCG-induced down-regulation of LH receptor (5). Hormonal manipulation of rats has been shown to cause changes in LHR mRNA expression (1–3, 12, 13). Using this paradigm, we have examined the relationship between LRBP activity and LHR mRNA expression. The data presented in this report (Figs. 5 and 6) clearly show that when the expression of LHR mRNA is low, the ovarian S-100 fractions yield higher levels of ribonucleoprotein complexes, establishing an inverse relationship between LHR mRNA expression and LRBP mRNA binding activity of LRBP. This inverse relationship suggests that LRBP might play a role in the accelerated degradation of LHR mRNA in vivo. This possibility is supported by the observation that LHR mRNA degradation is accelerated by the addition of LRBP in the in vitro decay assay (Figs. 3 and 4). We have found that LRBP binds to a polypyrimidine-rich bipartite sequence in the coding region of LHR mRNA (5’–202UCUCX–3’UCUCU220–3’) with an apparent dissociation constant of 10–19 M (5). LRBP could function by targeting an endonuclease to LHR mRNA either by direct interaction or by targeting the receptor mRNA to a particular location in the cytoplasm where mRNA degradation occurs. The increased LHR mRNA decay activity seen under in vitro conditions in the presence of LRBP is an indication that LRBP is one of the trans-acting factors involved in LHR mRNA degradation. It is possible that other proteins associated with the ribosomes might also participate in the decay process.

A number of cytoplasmic proteins, some of which shuttle between nucleus and cytoplasm, have been identified as either RNA-stabilizing or destabilizing trans-acting factors (14–17). Studies in a variety of systems have demonstrated the presence of binding sites for trans-acting factors in the coding region of mRNA (18–23) similar to that seen in this study. For example, the post-transcriptional regulation of tropoelastin mRNA in response to transforming growth factor β1 is very similar to the LHR mRNA regulation that we describe in this report. Similar to our system, tropoelastin mRNA contains an 18-nucleotide cis-acting motif near the 5’ end of the coding region, and a 50-kDa cytosolic protein interacts with this cis-acting motif causing destabilization of the mRNA (24). c-Fos and c-Myc mRNAs are some of the other well characterized mRNAs that contain binding sites in the coding region for trans-acting factors (19, 21, 22, 25, 26). The present studies clearly show that the LHR mRNA-binding protein LRBP, which binds to a region between nucleotides 188 and 228 in the open reading frame of LHR mRNA, plays a crucial role in the hormonal control of LHR mRNA stability. The mechanisms, which regulate the degradation of LHR mRNAs, are not clearly understood, although several mechanisms that involve deadenylation, decapping, and exonucleolytic and endonucleolytic degradation of eukaryotic mRNAs have been proposed (18, 27, 28). Regulated degradation of mRNA allows rapid cessation of protein synthesis without the need to alter transcription rates. The ability to regulate receptor protein expression at the level of mRNA degradation not involving changes in rate of transcription as demonstrated here provides a novel strategy for the control of gene expression under selective physiological or pharmacological states. The present study provides for the first time a novel

FIG. 6. LHR mRNA expression and RNA binding activity of LRBP during corpus luteum life span. Ovaries were collected from FMSG/hCG-primed rats on days 2, 4, 6, 8, 10, 12, and 14 of pseudopregnancy. A, LHR mRNA levels were obtained by Northern blot analysis. B, the Northern blot was normalized to 18S rRNA. C, the RNA binding activity of LRBP was measured by RNA electrophoretic mobility shift analysis using 50 μg of S-100 extract. D, the 6.7-kb LHR mRNA transcript and RNA electrophoretic mobility shift analysis bands were quantitated by densitometric scanning and expressed in arbitrary units.

LRBP was observed on day 10 of pseudopregnancy. The binding activity of LRBP started decreasing from day 10 but remained higher than the levels seen on days 6 and 8. Taken together, these results show that the LHR mRNA binding activity of LRBP is inversely related to LHR mRNA expression.

DISCUSSION

Differences in expression of the LH receptor observed during follicular development, ovulation, and luteinization involve concomitant changes in the steady-state levels of LH receptor mRNA (1, 2, 11–13). Previous studies have shown that during hCG-induced down-regulation of the LH receptor, the steady-state levels of LHR mRNA show a dramatic decline (2). Furthermore, it has been shown that the selective loss of LH receptor mRNA is because of increased mRNA degradation rather than decreased transcription (4).

We have recently identified a 50-kDa LHR mRNA-binding protein on native acrylamide gel designated as LRBP that is induced during down-regulation of the LH receptor (5). The present studies have examined the role of LRBP in LHR mRNA degradation during hCG-induced down-regulation of LH receptor. We have used an in vitro mRNA decay system to determine the effect of LRBP on the stability of LHR mRNA. In general, cell-free mRNA turnover systems afford many benefits for studying mRNA degradation. Although decay rates of mRNA in vitro generally occur at slower rates than in intact cells, the relative rates of turnover of different mRNAs are maintained. Moreover, the rates of mRNA degradation can be measured without the necessity of transcriptional repressing agents, which are generally nonspecific and cytotoxic. The in vitro

LH Receptor mRNA-binding Protein

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mechanism by which LHR mRNA is regulated by altering the rate of mRNA degradation.

REFERENCES

1. LaPolt, P. S., Oikawa, M., Jia, X. C., Dargan, C., and Hsueh, A. J. (1990) Endocrinology 126, 3277–3279
2. Hoffman, Y. M., Peegel, H., Sprock, M. J., Zhang, Q. Y., and Menon, K. M. J. (1991) Endocrinology 128, 388–393
3. Peegel, H., Randolph, J. J., Midgley, A. R., and Menon, K. M. J. (1994) Endocrinology 135, 1044–1051
4. Lu, D. L., Peegel, H., Mosier, S. M., and Menon, K. M. J. (1993) Endocrinology 132, 235–240
5. Kash, J. C., and Menon, K. M. J. (1998) J. Biol. Chem. 273, 10658–10664
6. Kash, J. C., and Menon, K. M. J. (1999) Biochemistry 38, 16889–16897
7. Ross, J. (1993) in RNA-Protein Interaction Protocols (Haynes, S. R., ed) pp. 459–476, Humana Press, Totowa, NJ
8. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
9. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
10. Golos, T. G., Miller, W. L., and Strauss, J. F., III (1987) J. Clin. Invest. 80, 886–899
11. Segaloff, D. L., Wang, H., and Richards, J. S. (1990) Mol. Endocrinol. 4, 1856–1865
12. Camp, T. A., Rahal, J. O., and Mayo, K. E. (1991) Mol. Endocrinol. 5, 1405–1417
13. Nakamura, K., Minegishi, T., Takakura, Y., Miyamoto, K., Hasegawa, Y., Ibuki, Y., and Igarashi, M. (1990) Biochem. Biophys. Res. Commun. 172, 786–792
14. Peng, S. S.-Y., Chen, C.-Y. A., Xu, N., and Shyu, A.-B. (1998) EMBO J. 17, 3461–3470
15. Klausner, R. D., Rouault, T. A., and Harford, J. B. (1993) Cell 72, 19–28
16. Fan, X. C., and Steitz, J. (1998) EMBO J. 17, 3448–3460
17. Burd, C. G., and Dreyfuss, G. (1994) Science 265, 615–621
18. Decker, C. J., and Parker, R. (1994) Trends Biochem. Sci. 19, 336–340
19. Bernstein P. L., Herrick, D. J., Prokipcak, R. D., and Ross, J. (1992) Genes Dev. 6, 642–654
20. Herrick, D., and Ross, J. (1994) Mol. Cell. Biol. 14, 2119–2228
21. Shyu, A.-B., Belasco, J. G., and Greenberg M. E. (1991) Genes Dev. 5, 221–231
22. Shyu, A.-B., Greenberg, M. E., and Belasco, J. G. (1989) Genes Dev. 3, 60–72
23. Veyrune, J. L., Carillo, S., Vie, A., and Blanchard, J. M. (1995) Oncogene 11, 2127–2134
24. Zang, M., Pierce, R. A., Wachi, H., Mecham, R. P., and Parks, W. C. (1999) Mol. Cell. Biol. 19, 7314–7326
25. Chen, C.-Y., You, Y., and Shyu, A.-B. (1992) Mol. Cell. Biol. 12, 5748–5757
26. Prokipcak, R. D., Herrick, D. J., and Ross, J. (1994) J. Biol. Chem. 269, 9261–9269
27. Ross, J. (1995) Microbiol. Rev. 59, 423
28. Sachs, A. (1993) Cell 74, 413–421
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