Identification of a Hormonally Regulated Luteinizing Hormone/Human Chorionic Gonadotropin Receptor mRNA Binding Protein

INCREASED mRNA BINDING DURING RECEPTOR DOWN-REGULATION*

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John C. Kash‡ and K. M. J. Menon§

From the Departments of Biological Chemistry and Obstetrics/Gynecology, University of Michigan Medical School, Ann Arbor, Michigan 48109-0617

To elucidate the molecular events associated with the regulation of luteinizing hormone/human chorionic gonadotropin (LH/hCG) receptor mRNA stability during hCG-induced receptor down-regulation, we have identified an LH/hCG receptor-specific mRNA binding protein. Proteins were isolated from control and down-regulated rat ovary and were incubated with in vitro transcribed RNAs corresponding to the full-length LH/hCG receptor, as well as 5'- and 3'-truncated receptor RNA forms. Resultant ribonucleoprotein complexes were analyzed by RNA gel mobility shift. A prominent M, 50,000 ribonucleoprotein complex was identified with the following characteristics: 1) specificity for LH/hCG receptor open reading frame sequences located between nucleotides 102 and 282; 2) lack of competition by nonspecific RNAs; 3) a 3-fold increase in RNA binding activity during hCG-induced receptor down-regulation; and 4) limited tissue expression. This report describes the first evidence of an LH/hCG receptor mRNA binding protein, which we term LRBP-1, for luteinizing hormone receptor RNA binding protein-1. This protein is a candidate for a trans-acting factor involved in the hormonal regulation of LH/hCG receptor mRNA stability in rat ovary.

Interaction of luteinizing hormone (LH),1 or its human placental counterpart human chorionic gonadotropin (hCG), with its receptor is a key event in the regulation of steroidogenesis in the mammalian ovary. The LH/hCG receptor belongs to the family of G,-protein-coupled receptors that mediate their biological effects through cAMP (1). LH/hCG receptors expressed on rat ovarian granulosa cells of preovulatory follicles and luteal cells are greatly diminished after an endogenous preovulatory LH surge or by the administration of a pharmacological dose of hCG (reviewed in Ref. 2). Our laboratory has demonstrated that the decline in cell surface LH/hCG receptor number that occurs during hCG-induced down-regulation is paralleled by a specific loss of LH/hCG receptor mRNA (3). Following the injection of a pharmacological dose of hCG in female rat, a rapid decline in the steady state levels of all four of the LH/hCG receptor mRNAs (6.7, 4.4, 2.6, and 1.8 kb) is seen within 12 h, with complete loss of detectable receptor mRNAs by 24 h (3). This selective loss is followed by a recovery of mRNA expression between 24 and 48 h (3). We have further reported that loss of receptor mRNA does not result from decreased transcription, but rather occurs post-transcriptionally, with an approximate 3-fold decrease in receptor mRNA half-life (4).

It has been well established that the expression of specific, highly regulated mRNAs like c-fos, c-myc, and the β-adrenergic receptor are controlled, at least in part, at the level of mRNA degradation (5, 6). In the majority of instances of post-transcriptional regulation of mRNA, the changes in stability of a particular mRNA appear to result from changes in the binding of specific proteins to defined sequences and/or structures in the target mRNA. The RNA sequences recognized by regulatory proteins often are located within a discrete region(s) of the mRNA as follows: the 5'-untranslated region (UTR) in the case of ferritin, the open reading frame of tubulin, c-fos, c-myc, or the 3'-UTR of the β-adrenergic receptor and GM-CSF (6–11). Highly regulated mRNAs can also contain determinants for the control of stability in a combination of regions of the mRNA, as manifested in both c-fos and c-myc, which contain regulatory sequences in both the coding region and the 3'-UTR (12, 13). The ability to regulate protein expression at the level of mRNA degradation provides a fine control mechanism for the proper expression of genes involved in growth, differentiation, and development.

In the present work, we have identified and characterized a protein from pseudopregnant rat ovary which displays specificity for LH/hCG receptor mRNA using RNA gel mobility shift analysis. This study describes the characterization of this mRNA binding protein with respect to LH/hCG receptor mRNA sequence recognition, hormonal regulation, and tissue specificity.

MATERIALS AND METHODS

Animals and Tissues—Pseudopregnancy was induced in 21-day-old Sprague-Dawley female rats by a subcutaneous injection of 50 IU pregnant mare serum gonadotropin (Calbiochem), followed by 25 IU human chorionic gonadotropin (hCG; Sigma) 56 h later (14). LH/hCG receptor down-regulation was induced by the injection of 50 IU hCG on the 5th day of pseudopregnancy. Ovaries and other tissues were collected at the indicated times and were either processed immediately or were stored in liquid nitrogen.

Preparation of Tissue Extracts—Tissues were homogenized at 4 °C in a buffer containing 10 mM HEPES, pH 7.9, 0.5 mM MgCl2, 50 mM KCl, 50 μM EDTA, 2.5–5 mM dithiothreitol, and 10% glycerol. The homoge-
nization buffer additionally contained the following protease inhibitors: 1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride-hydrochloride (Boehringer Mannheim), 1 mM benzamidine, 1 mM leupeptin, and 1 mM EDTA. The homogenates were centrifuged at 105,000 × g for 90 min at 4 °C. The supernatants were collected and were stored on ice for immediate use. Proteins were quantified by bichinchoninic acid assay (Pierce).

Preparation of Templates for in Vitro Transcription—All constructs were cloned into the plasmid pscript II SK+ (Stratagene). pscript SK+ cDNA contains the full-length LH/hCG receptor coding region was obtained from Dr. William Moyle at the Robert Wood Johnson (Rutgers) Medical School (15). The 2.2 LH/hCG receptor cDNA was ligated into pscript II between XbaI and BamHI sites and was used to generate LR:1–1401 RNA (see Fig. 2). LH/hCG receptor cDNAs used as templates for the in vitro transcription of RNAs containing varying 3′ ends were prepared by truncation of the 2.2-kb cDNA with restriction endonucleases as follows: LR:1–1401 RNA by truncation with AccI and LR:1–711 with ApaI. A 609-base pair cDNA containing 102–711 of the LH/hCG receptor coding region was prepared by directional cloning of a fragment of the 2.2-kb cDNA digested with PstI and ApaI to prepare LR:1–711 RNA. This cDNA was further truncated at the 3′ end using BglII or DdeI to obtain LR:1–468 or LR:1–292 RNA, respectively. An LH/hCG receptor cDNA containing nucleotides 711–1401 was prepared by directional cloning of an AccI, ApaI fragment of the 2.2-kb cDNA, to generate LR:711–1401 RNA. The nomenclature used throughout this report to describe LH/hCG receptor RNAs designates nucleotide 1 as the first nucleotide transcribed in the 5′-untranslated region of the 2.2-kb cDNA. A full-length human glyceraldehyde-3-phosphate dehydrogenase mRNA was obtained from ATCC (16).

In Vitro Transcription—Unlabeled and [α-32P]UTP-labeled RNAs were synthesized from linearized cDNA templates essentially as described by Melton et al. (17). Transcriptions of linearized plasmids were performed with T3 or T7 RNA polymerase (Life Technologies, Inc.) at 30–37 °C in the presence of 40 units of RNasin (Promega), and 50–150 μCi (800 Ci/μmol) of [α-32P]UTP (NEN Life Science Products). Following transcription, RNAs were treated with RNase-free DNase I and were extracted with water-saturated phenol and chloroform/isoamyl alcohol (49:1). Unincorporated nucleotides were separated from RNA by the addition of 2 units of RNase T1 (Boehringer Mannheim) at 37 °C for 10 min (18, 19). Unprotected radiolabeled RNA was then degraded by the addition of 2 units of RNase T1 (Boehringer Mannheim) at 37 °C for 30 min. When indicated, unlabeled competitor RNAs were also added to the binding assay. Samples were then incubated with heparin at a final concentration of 5 mg/ml for 10 min on ice. The RNA-protein complexes were resolved by 8% native-polyacrylamide gel electrophoresis at 4 °C. The gel was then dried and exposed to Kodak XAR film and visualized by autoradiography.

Northern Blot Analysis—Total RNA was extracted using a previously described procedure (20). Ovaries were homogenized in a solution of guanidine isothiocyanate, acidified with 2 M sodium acetate, pH 4.0, and, and extracted with water-saturated phenol and chloroform/isoamyl alcohol (49:1). RNA was precipitated at −20 °C using 3 volumes of ethanol and was quantified by UV absorbance spectroscopy. Northern blot hybridization analysis was performed essentially as described by Maniatis and co-workers (21). Briefly, aliquots of total RNA were separated by electrophoresis in 1.2% agarose-formaldehyde gels and transferred to nitrocellulose membranes using 10× saline/sodium citrate buffer, pH 7 (SSC). Integrity of the RNA was determined prior to transfer to nitrocellulose by ethidium bromide staining. Blots were then heated at 80 °C under vacuum for 2 h and were prehybridized at 42 °C for 2 h in a solution containing salmon sperm DNA (0.5 mg/ml) and 2× hybridization buffer (1.5 × NaCl, 0.1 × TES, pH 7.1, 0.1 × EDTA, 2× Denhardt’s) diluted 1:1 with deionized formamide. The hCG receptor cDNA probe was radiolabeled using [α-32P]dCTP (ICN) and the Klenow fragment of DNA polymerase and was hybridized to blots overnight at 42 °C in fresh hybridization buffer using 2 × 106 cpm of the labeled probe. Blots were then washed four times with 2× SSC containing 0.1% SDS for 10 min each at room temperature and once at 60 °C for 30 min. The washed blots were exposed to Kodak XAR film.

RESULTS

Analysis of Steady State LH/hCG Receptor mRNAs during in Vivo hCG-induced Down-regulation—Previous studies from our laboratory have demonstrated that during hCG-induced down-regulation of the LH/hCG receptor in rat ovary, the steady state levels of LH/hCG receptor RNAs dramatically decline, with an approximate 3–4-fold decrease in mRNA half-life (3, 4). The temporal changes of the steady state levels of the ovarian LH/hCG receptor transcripts that occurred during hCG-induced down-regulation were therefore examined by Northern blot hybridization analysis. Total RNA was isolated at 0, 6, 12, 24, 48, and 72 h post-injection from ovaries of pseudopregnant rats which were injected with saline (control) or hCG (down-regulated). Relative abundance of LH/hCG receptor mRNAs was compared by Northern blot hybridization analysis using a radiolabeled carboxyl-terminal LH/hCG receptor cDNA probe, and the results are shown in Fig. 1A. Under control conditions (lanes 1–6), the steady state levels of the 6.7-4.4-, 2.6-, and 1.8-kb LH/hCG receptor transcripts remained relatively constant from 0 to 72 h. In contrast, injection of hCG markedly affected the amount of receptor mRNA, with a substantial decline occurring between 6 and 12 h post-injection (lanes 7 and 8). By 24 h post-injection, no steady state LH/hCG receptor mRNA was detectable (lane 9). Recovery of steady state levels of receptor mRNA occurred by 48–72 h (lanes 10 and 11). Integrity of the RNA, as determined by staining of the 28 S and 18 S rRNAs with ethidium bromide, is shown in Fig. 1B. The abundance of all four mRNAs (6.7, 4.4, 2.6, and 1.8 kb) declined during down-regulation and returned to near control levels by 72 h, which suggested that the turnover of the four receptor transcripts could be occurring via a common mechanism(s). Since specific mRNA binding proteins have been implicated in selective mRNA degradation, we investigated whether any LH/hCG receptor mRNA binding proteins were expressed in rat ovary.

Determination of Two Open Reading Frame LH/hCG Recep-
tor RNA Binding Proteins in Rat Ovary—To investigate for the presence of LH/hCG receptor mRNA binding proteins, we employed gel mobility shift analysis by incubating ovarian S100 protein extracts with several radiolabeled LH/hCG receptor RNAs. The LH/hCG receptor RNAs used in the mobility shift assays are depicted in Fig. 2. Ovaries were isolated 6 h post-hCG injection. This time interval was initially chosen because LH/hCG receptor degradation was first observable 6–12 h post-injection, as demonstrated in Fig. 1A. We expected any RNA binding activity should be observable at the onset of accelerated receptor mRNA turnover, thus the 6-h time interval was chosen. Subsequent experiments were performed with ovaries isolated at longer times (8 and 12 h) post-injection (see Figs. 3B–7). These data demonstrated that binding of LH/hCG receptor RNA by LRBP-1 and LRBP-2 was observed at these later times of receptor down-regulation and achieved maximal binding at 12 h post-injection. Radiolabeled RNAs corresponding to distinct regions of the 5'-UTR and the open reading frame were incubated in the absence or presence of 50 μg of S100 prepared from ovaries 6 h following down-regulation and kidney isolated from pregnant (day 10) rats in the presence of 5 μg of tRNA, 2.5 mM dithiothreitol, 20 mM phosphocreatine, 40 units of RNasin (Promega) with the addition of 2 mM ATP at 30 °C for 10 min (19). In later experiments, phosphocreatine and ATP were not included, as they did not have any effect on the RNA gel mobility shift assay. Samples were processed as described under “Materials and Methods” and were resolved by 8% native-polyacrylamide gel electrophoresis. As shown in Fig. 3A, RNA gel mobility shift analysis was performed by incubation of down-regulated ovarian S100, pregnant rat kidney S100, or no protein (as indicated in the figure) with 1 × 10⁶ cpm radiolabeled RNAs: LR:1–2131 (lanes 1–3), LR:1–1401 (lanes 4–6), LR:711–1401 (lanes 7–9), LR:1–711 (lanes 10–12), LR:102–711 (lanes 13–15), or full-length glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (lanes 16–18). B, autoradiogram of RNA gel mobility shift analysis performed by incubation of 1 × 10⁶ cpm LR:1–2131 RNA (lanes 1–3), LR:102–711 RNA (lanes 4–6), or LR:102–282 RNA (lanes 7–9) with no protein (lanes 1, 4 and 7), 25 μg of control ovarian S100 (lanes 2, 5, and 8), or 25 μg of hCG down-regulated (12 h) ovarian S100 (lanes 3 and 6 and 9). These figures are representative of five and three separate experiments, respectively. C, control protein; D, down-regulated protein.

strates that these species represent protein-RNA complexes and not artifacts arising from RNase T1-resistant RNA-RNA duplexes. A larger molecular mass species of approximately 165 kDa was also observed (lanes 1, 2, 4, 5, 7, 13, and 16) but was not further examined because it was not specific to the ovary. The radiolabeled species with Mr <40,000 represent RNase T1 digestion products, as they were observed in samples incubated in the absence of S100.

To characterize further LRBP-1 and LRBP-2, the RNA bind-
ing specificity of the S100 fractions isolated from saline-treated (control) and hCG down-regulated ovaries was examined. The ovaries were collected at 12 h following hCG treatment, the time at which the steady state levels of receptor mRNA were markedly reduced (Fig. 1A). Briefly, 1 × 10^5 cpm LR:102–282 RNA was incubated in the absence (lanes 1, 4, and 7) or presence of equal concentrations of S100 from control (lanes 2, 5 and 8) or down-regulated ovaries (lanes 3, 6, and 9). Samples were then treated with RNase T1 and separated by 8% native-polyacrylamide gel electrophoresis. The results shown in Fig. 3B demonstrate that the 50-kDa LRBP-1 RNA binding activity increased in the hCG-treated ovarian S100 compared with untreated controls. Additionally, this induction of RNA binding was observed using all LH/hCG receptor RNAs containing the nucleotides 102–282. These results demonstrated that specific RNA binding proteins were expressed in the rat ovary which recognized receptor RNA sequences contained between nucleotides 102 and 282. Furthermore, the 50-kDa LRBP binding activity increased following hCG treatment, whereas the 45-kDa LRBP-2 complex did not exhibit hCG responsiveness.

**Tissue Expression Pattern of LRBP-1 and LRBP-2**—The tissue expression pattern of the LRBP-1 and LRBP-2 was then determined by performing RNA gel mobility shift analysis with proteins isolated from different tissues collected from down-regulated rats. As shown in Fig. 4, radiolabeled LR:102–468 RNA (1 × 10^5 cpm) was incubated with 50 µg of S100 isolated from spleen (lane 1), lung (lane 2), liver (lane 3), kidney (lane 4), heart (lane 5), adrenal gland (lane 6), and ovary (lane 7). Unbound RNA was then degraded by the addition of 2 units of RNase T1, and the samples were then resolved by 8% native-polyacrylamide gel electrophoresis. The 50-kDa LRBP-1 RNP complex was seen only when the radiolabeled LH/hCG receptor RNA was incubated with S100 isolated from spleen and ovary (lanes 1 and 7). The functional significance of the presence of an approximately 50-kDa RNP complex in samples containing spleen S100 (lane 1) has yet to be determined. It is possible that LRBP-1 is expressed in the spleen or that this species represents a similarly sized RNA binding protein that can interact with LH/hCG receptor RNA in vitro. Adrenal gland did not appear to express either LRBP-1 or LRBP-2 (lane 6). Interestingly, the 45-kDa LRBP-2 complex was detected in ovary, heart, kidney, liver, lung, and spleen. In contrast to Fig. 3, the 45-kDa LRBP-2 RNP complex was visible in the S100 of kidney isolated from 12-h down-regulated pseudopregnant rat but was not present in S100 isolated from day 10 pregnant rat kidney. The difference in appearance of the LRBP-2 RNP complex is likely due to the differences in treatments of the two groups. We believe the 45-kDa species may represent a ubiquitous RNA binding protein originating in the tissue itself or present in blood contained within the vasculature of the tissue.

**Concentration-dependent RNP Complex Formation**—To examine the effect of protein concentration on the intensity of the LRBP-1 and LRBP-2 RNP complexes, gel shift analysis was performed using radiolabeled LR:102–282 RNA incubated with increasing concentrations of down-regulated ovarian S100. As shown in Fig. 5, LR:102–282 RNA (1 × 10^5 cpm) was incubated with increasing concentrations (2.5, 5, 10, 25, or 50 µg) of ovarian S100, as described previously. Unbound RNA was degraded, and the samples were resolved by 8% native-polyacrylamide gel electrophoresis. Incubation of LR:102–282 RNA with ovarian S100 generated the 50- and 45-kDa RNP complexes in a concentration-dependent manner. These data demonstrated the intensities of the radiolabeled LRBP-1 and LRBP-2 RNP complexes were dependent on protein concentration in the RNA binding reaction.

**Determination of Changes in LH/hCG Receptor mRNA Binding by LRBP-1 during hCG-induced Down-regulation**—To determine the change in binding activity of LRBP-1 during hCG-induced receptor mRNA destabilization (see Fig. 3B), RNA gel shift analysis was performed using saline-injected control and hCG down-regulated ovarian S100. Reporte RNA mobility shift reactions were carried out with either control or down-regulated ovarian S100. Briefly, LR:102–282 RNA (1 × 10^5 cpm) was incubated with 50 µg of S100 isolated from control (saline-treated) (lanes 1–4) and down-regulated (lanes 5–8) rat ovaries. A 12-h post-hCG injection time interval was chosen because it corresponds to the most acute phase of LH/hCG receptor mRNA decay, as shown in Fig. 1A. Samples were then treated with RNase T1 and were separated by 8% native-polyacrylamide gel electrophoresis. As shown in Fig. 6A, the LH/hCG receptor mRNA binding activity of LRBP-1 increased following exposure to hCG, whereas the binding of LRBP-2 remained constant. The intensities of the LRBP-1 and LRBP-2 RNP complexes in Fig. 6A were quantified by densitometry and are shown in Fig. 6B. This analysis revealed that the binding activity of LRBP-1 increased approximately 3-fold over control levels 12 h following injection with hCG. This induction of LRBP-1 RNA binding activity correlated well with a 3-fold decrease in receptor mRNA half-life previously reported (7). The LRBP-2 RNP complex was not regulated by hCG, further suggesting that this protein may not represent an LH/hCG receptor-specific mRNA binding protein involved in the regul-
lation of receptor mRNA stability. The demonstration that LRBP-1 RNP complex formation was regulated in vivo by hCG is consistent with the hypothesis that the hormonally controlled binding of LRBP-1 to LH/hCG receptor mRNA may be involved in the control of receptor mRNA turnover.

**Analysis of RNA Binding Specificity—**To examine the specificity of LRBP-1 and LRBP-2 for LH/hCG receptor RNA, gel shift analysis was performed by incubating ovarian S100 with fixed concentrations of radiolabeled receptor RNA in the presence of increasing concentrations of unlabeled competitor RNAs containing different LH/hCG receptor open reading frame sequences. As shown in Fig. 7, $1 \times 10^5$ cpm radiolabeled LR:102–468 RNA (9 ng) was incubated with 50 μg of down-regulated ovarian S100 with 1 $\times 10^5$ cpm LR:102–282 RNA. This figure is representative of three separate experiments.

**FIG. 5.** Concentration-dependent RNP complex formation. Autoradiogram of RNA gel mobility shift analysis performed by incubation of increasing concentrations 2.5 μg (lane 1), 5 μg (lane 2), 10 μg (lane 3), 25 μg (lane 4), or 50 μg (lane 5) of hCG down-regulated (12 h) ovarian S100 with $1 \times 10^5$ cpm LR:102–282 RNA. This figure is representative of three separate experiments.

**FIG. 6.** LH/hCG receptor RNA binding activity of LRBP-1 and LRBP-2 during hCG-induced down-regulation. Ovaries were collected from four saline-injected (Control) and four hCG down-regulated (D-Reg.) (12 h) rats. S100s were then prepared from the pooled control and pooled hCG down-regulated ovaries. A, autoradiogram of RNA gel mobility shift analysis. RNA binding reactions were carried out in replicates of four by incubating of $1 \times 10^5$ cpm LR:102–282 RNA with 50 μg of S100 from the pooled saline-treated control (lanes 1–4) or pooled hCG down-regulated (lanes 5–8) ovaries. B, bar graph representing the mean densitometric scans ± S.D. of the LRBP-1 and LRBP-2 complexes shown in A. The LRBP-1 and LRBP-2 complexes in each lane were quantified, and the mean ± S.D. of the control (C) and down-regulated (DR) treatment groups are presented. This figure is representative of three separate experiments.

complex seen in Fig. 7 showed a similar pattern of competition with the specific and nonspecific unlabeled RNAs. The increase in intensity of the LRBP-1 and LRBP-2 RNP complexes observed in lanes 6–9 is probably due to stabilization of the radiolabeled
The data presented in this report demonstrate that the protein LRBP-1 specifically recognizes and binds sequences within the open reading frame of the LH/hCG receptor mRNA. Several studies have demonstrated that sequences in the open reading frame of an mRNA can regulate mRNA stability. For example, in the presence of excess tubulin monomers, the amino-terminal tetrapeptide encoded by β-tubulin mRNA provides a signal for the rapid autoregulated decay of that mRNA (8, 24). Cleveland and colleagues (25, 26) were further able to show that translation of the first 41 codons was required for the destabilization of polysomal β-tubulin mRNA. Studies on c-fos have also demonstrated the presence of two destabilizing regions in the open reading frame, in addition to the AU-rich element in the 3′-UTR (12). More recent studies have demonstrated that translation through the c-fos open reading frame destabilizing sequences is required for induction of mRNA decay (27). A third example is the stability of c-myc mRNA, which is influenced by a destabilizing pyrimidine-rich sequence in the carboxyl-terminal open reading frame termed the coding region determinant (CRD) (10). Ross and colleagues (28, 29) have further demonstrated that CRD is bound by a 70-kDa protein termed CRD-BP which selectively binds the destabilizing CRD RNA sequences, protecting the RNA from endonucleolytic cleavage and thereby helping to stabilize the mRNA.

In the rat ovary, LH/hCG receptor precursor mRNA is processed into four mRNAs of 6.7, 4.4, 2.6, and 1.8 kb (30). The splicing events generating the 6.7-, 4.4-, and 2.6-kb transcripts are primarily limited to the 3′-UTR, and all three transcripts contain the 2.1-kb open reading frame encoding the full-length receptor, whereas the 1.8-kb transcript is believed to contain the amino-terminal ligand binding domain and the carboxyl-terminal intracellular tail (31, 32). Interestingly, during hCG-induced receptor down-regulation, all four ovarian LH/hCG receptor transcripts appear to be coordinately destabilized (see Fig. 1A). These results suggest that if the destabilization of all four LH/hCG receptor mRNAs occurs by a similar mechanism, the RNA sequences involved in this process would be expected to be common to all four transcripts. The LRBP-1 binding site located within nucleotides 102–282 is indeed common to the four different LH/hCG receptor transcripts in rat ovary. The selective recognition and binding of LRBP-1 to this common LH/hCG receptor mRNA sequence are consistent with the hypothesis that LRBP-1 may be involved in the control of LH/hCG receptor mRNA stability during hCG-induced down-regulation. The nucleotide composition of the 180-base pair LR:102–282 RNA, containing the LRBP-1 binding site, includes a 60-nucleotide domain that is approximately 50% cytosine. In gel mobility shift assays where homo-ribopolymers were used as competitors, only polycytidylic acid was able to effectively compete for LRBP-1 and LRBP-2 binding (data not shown). Thus, LRBP-2 might recognize the cytosine-rich region of the LR:102–468 RNA rather than the specific LH/hCG receptor sequences.

Taken together, these results demonstrate that the RNA binding protein LRBP-1 fulfills the requirements of specificity and hormonal regulation that would be expected for a protein involved in the hormonal control of LH/hCG receptor mRNA turnover.

DISCUSSION

Our previous studies have shown that hCG-induced down-regulation of the LH/hCG receptor is accompanied by a dramatic decline of receptor mRNA (3, 4). Furthermore, we were able to show that the selective loss of LH/hCG receptor mRNA is due to increased mRNA degradation and not decreased gene transcription (4, 22). The identification and characterization of trans-acting stabilizing/distabilizing factors which recognize and bind specific LH/hCG receptor mRNA sequences provide important information that should shed light on the understanding of post-transcriptional mechanism(s) involved in hCG-induced receptor down-regulation. Recent studies have shown that multiple, and perhaps redundant, control mechanisms are utilized for the regulation of mRNA stability (reviewed in Ref. 23).

The functional consequences of the association of LRBP-1
with LH/hCG receptor mRNA described in this report have yet to be determined. Additionally, the relationship between LRBP-1 and the less abundant LRBP-2 is not clear. It is possible that LRBP-2 is a poly(rC)-specific, ubiquitous RNA binding protein that interacts with LR:102–282 RNA due to the high cytosine content of that RNA. However, in this report, we have demonstrated specific, hCG-regulated binding of LRBP-1 to sequences contained within nucleotides 102–282 of LH/hCG receptor mRNA. The LH/hCG receptor mRNA binding protein described here fulfills the requirements of RNA specificity and hormonal regulation which would be expected for a protein involved post-transcriptional regulation of the LH/hCG receptor. These data are consistent with the supposition that the hormonally regulated binding of LRBP-1 to receptor open reading frame sequences influences the selective destabilization of receptor mRNA which acts as a fine control of LH/hCG receptor expression.

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