Isolation and Characterization of a Novel trans-Factor for Luteinizing Hormone Receptor mRNA from Ovary*

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Regulation of mRNA turnover is one of the major control mechanisms of gene expression in all organisms. mRNA half-lives are influenced by the interaction of various cytoplasmic proteins (trans-acting factors) with regulatory regions (cis-acting elements) in the mRNA, forming ribonucleoprotein (RNP) complexes (10). The formation and disruption of RNP complexes in response to various cellular stimuli mainly controls the turnover of cytoplasmic mRNA. Studies have indicated the presence of cis-acting regulatory elements in the 5′-untranslated region, coding region, and 3′-untranslated region of mRNA (10). A number of cytoplasmic trans-acting factors, some of which shuttle between the nucleus and cytoplasm, have been identified as mRNA-stabilizing, destabilizing, or translational repressor proteins (11–16). c-Fos, c-Myc, tropoelastin, thymidylate synthase, and dihydrofolate reductase are some of the mRNAs containing regulatory elements in the coding region for trans-acting factors (17–24).

We have identified a LHR mRNA-binding protein in rat and human ovary, designated as LRBP, which binds to a polypyrimidine-rich bipartite sequence in the coding region of LHR mRNA with high affinity (25). Further studies have demonstrated that in an in vitro reconstituted mRNA decay system, the addition of partially purified LRBP caused accelerated degradation of LHR mRNA (26). In the present study, we have purified the LHR mRNA-binding protein from rat ovaries, established its identity as mevalonate kinase by N-terminal microsequencing, and subsequently cloned and characterized the expressed protein in 293 cells. We also show that the LHR mRNA binding activity of mevalonate kinase is inhibited by its substrates, mevalonate and ATP, in a concentration-dependent manner. Furthermore, depletion of cellular levels of mevalonate resulted in increased binding of LRBP to LH receptor mRNA.

MATERIALS AND METHODS

Chemicals—Highly purified human chorionic gonadotropin (CR 127) was a gift from the Center for Population Research (NICHD, National Institutes of Health), through the National Hormone and Pituitary Program. Pregnant mare serum gonadotropin was obtained from Calbiochem. The Macro-Prep high S support column was from Bio-Rad. EDTA-free protease inhibitor mixture tablets, RNase T1, Fugene 6 reagent, and Quick spin columns (G-50-Sephadex) for radiolabeled RNA.
purification were purchased from Roche Applied Science. [α-32P]UTP was obtained from PerkinElmer Life Sciences. mRNA Machine Kit and MAXScript Kit were the products of Ambion (Austin, TX). SuperScript One-Step RT-PCR with PLATINUM Taq system was purchased from Invitrogen. RNasin was obtained from Promega (Madison, WI). Centriplus YM-10, Centricon YM-10, Microcon YM-10 microconcentrators, and the Ultrafree-DA kit were products of Millipore Corp. (Bedford, MA). Anti-N-terminal mevalonate kinase IgG preparation was a gift from Dr. Skaidrite K. Krišjans (Department of Biology, San Diego State University, San Diego, CA) (27). This antibody was raised against the first 15 amino acids of the amino-terminal portion of rat mevalonate kinase (NH2-MLSEVLLVSAPGKVI-COOH). Protein A/G (Bedford, MA). Anti-N-terminal mevalonate kinase IgG preparation. Centriplus YM-10, Centricon YM-10, Microcon YM-10 microconcentrators, and the Ultrafree-DA kit were products of Millipore Corp. (Bedford, MA). Anti-N-terminal mevalonate kinase IgG preparation was a gift from Dr. Skaidrite K. Krišjans (Department of Biology, San Diego State University, San Diego, CA) (27). This antibody was raised against the first 15 amino acids of the amino-terminal portion of rat mevalonate kinase (NH2-MLSEVLLVSAPGKVI-COOH). Protein A/G (Bedford, MA). Anti-N-terminal mevalonate kinase IgG preparation. WI). RNasin was obtained from Promega (Madison, WI). Elution and Renaturation of Proteins from SDS-PAGE—LHR mRNA-binding protein (LRBP) was eluted from SDS-polyacrylamide gel and renatured according to the method developed by Ossipow et al. (28) with a minor modification. Partially purified LRBP preparation was treated with Sambrook's sample buffer at 37 °C for 10 min and separated on a 10% SDS-polyacrylamide gel under constant voltage (100 V) for 40 min. The proteins were then electroblotted onto nitrocellulose membrane using 50 mM Tris buffer (pH 7.4, 75 mM NaCl, 0.1% SDS, and 50% methanol) with a Teflon pestle and incubated for 2 h at 37 °C. After incubation, the samples were centrifuged at 10,000 × g for 5–10 min at 4 °C to sediment the residual polyacylamide. The supernatants containing the proteins were collected and were then buffer-exchanged with buffer A containing 50 mM KCl using Centricym YM-10 microconcentrators. The protein concentration was determined by BCA. Elution and Renaturation of Proteins from SDS-PAGE—LHR mRNA-binding protein (LRBP) was eluted from SDS-polyacrylamide gel and renatured according to the method developed by Ossipow et al. (28) with a minor modification. Partially purified LRBP preparation was treated with Sambrook's sample buffer at 37 °C for 10 min and separated on a 10% SDS-polyacrylamide gel under constant voltage of 150 V. The gel strips containing the fractionated proteins were cut with a razor blade and placed in separate 1.5-ml Eppendorf tubes. The gel pieces were then homogenized in elution-renaturation buffer (1% Triton X-100, 20 mM Hepes, pH 7.4, 0.5 mM MgCl2, 50 mM NaCl, 100 mM KCl, and 50% ethanol) with a Teflon pestle and incubated for 2 h at 37 °C. After incubation, the samples were centrifuged at 10,000 × g for 5–10 min at 4 °C to sediment the residual polyacylamide. The supernatants containing the proteins were collected and were then buffer-exchanged with buffer A containing 50 mM KCl as (described above) to remove Triton X-100 and concentrated using Microcon centrifugal filter devices as described by the manufacturer. Northwestern Analysis—Northwestern analysis was performed as described by Holick and Liebhaber (29). The cytoplasmic proteins (S100) from LHR down-regulated rat ovaries and partially purified LRBP were separated on 10% SDS-PAGE under constant voltage (100 V) for 40 min. The proteins were then electroblotted onto nitrocellulose membrane using 50 mM Tris buffer containing 14.5 mM glycine and 20% methanol, at a constant current of 150 mA for 1 h. After electroblotting, the membrane was briefly rinsed in phosphate-buffered saline followed by a 2-h incubation in renaturing buffer (10 mM Tris-HCl, pH 7.4, 50 mM NaCl, 1 mM EDTA, pH 8.0, 1% SDS, and 50% methanol) with gentle shaking for renaturation of proteins. The blot was overlaid with [32P]-labeled LRBP binding sequence (LBS) of LHR mRNA (~50,000 cpm/ml) in hybridization buffer (renaturing buffer containing 20 μg/ml tRNA and 5 μg/ml heparin) and incubated for 2 h at room temperature with gentle rocking. The membrane was then washed three times (5–10 min each) in washing buffer until the background reached background levels as indicated in the figure legends. Samples were then incubated with heparin at a final concentration of 5 μg/ml for 10 min on ice to decrease nonspecific binding. The RNA-protein complexes were resolved by 5% native polyacrylamide (70:1) gel electrophoresis at 4 °C. The gel was then dried and exposed to Eastman Kodak Co. X-Omat AR film and visualized by autoradiography. RNA supershift analysis was performed by adding the antibodies to the binding reaction after the RNP complex formation and incubating the reaction mixture at room temperature for 1 h. Then the complexes were resolved on a 5% native polyacrylamide (70:1) gel. Competition with ATP, UTP, and mevalonate was performed with overexpressed mevalonate kinase from 293 cells by including ATP, UTP, and mevalonate in the binding reaction in molar levels as indicated in the figure legends.

Immunodepletion of Mevalonate Kinase from Partially Purified LRBP—Partially purified LRBP preparation was incubated with specific antibody for rat mevalonate kinase or nonspecific antibody for high density lipoprotein-binding protein (HBP) for 2 h in an end-over-end shaker at 4 °C. Protein A/G-agarose beads were then added, and incubation was continued for 2 h. The reaction mixtures were centrifuged, and the supernatants were then collected. Protein concentrations were determined by measuring absorbance at 260 nm. The mevalonate kinase cDNA was generated using the SuperScript One-Step RT-PCR with PLATINUM Taq system. The oligonucleotide primers were synthesized by Invitrogen, and their sequences were as follows (the underlined bases are not part of the target gene sequence and represent unique restriction endonuclease sites for cloning purposes): the mRNA sequence (the lower strand) joining the parts of the MVMV4 (mammalian virus expression vector) frameset primer 5′-GGGGTACCTCAGGAGCCATGTTGTCAGA-3′; and antisense primer 5′-CCATCGATGTCAGAGCC-GAGGGTGTCCGACAGG-3′. The reaction was performed in a final volume of 50 μl as described by the manufacturer using PTC-100 programmable thermal cycler (MJ Research Inc.). The reaction conditions were as follows: reverse transcription at 42 °C for 1 h; denaturation at 94 °C for 2 min; PCR amplification (40 cycles), denaturation at 94 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 1 min. The reverse transcriptase-PCR product was resolved in a 0.8% agarose gel stained with ethidium bromide. The gel containing the mevalonate kinase cDNA was then recovered from the gel using the Ultrafree-DA kit. The sequence of the MVMV4 was confirmed by sequencing at the Biomedical Sequencing Core facility at this university. It was cloned into the KpnI/ClaI site of the mammalian expression vector pCMV4 (31).
Overexpression of Rat Mevalonate Kinase in 293 Cells—Human embryonic kidney cells (293 cells) were transfected with mevalonate kinase DNA cloned into the pCMV4 vector using Fugene 6 reagent as described by the manufacturer. Cells were collected 24 and 48 h after transfection, and the cytoplasmic proteins (S100) were prepared as described before (9). The S100 fractions were then analyzed for mevalonate kinase by Western blot analysis, and the RNA binding activity was determined by RNA electrophoretic mobility shift analysis.

Western Blot Analysis—Proteins were separated on a 10% SDS-PAGE and transferred onto nitrocellulose membrane using 25 mM Tris buffer containing 192 mM glycine and 20% methanol (pH 8.3) for 1 h at 4°C. The nitrocellulose membrane was immersed in 5% nonfat dried milk in phosphate-buffered saline containing 0.05% Tween 20 overnight at 4°C. Rat mevalonate kinase was detected using a rabbit polyclonal IgG preparation (this preparation will be described as anti-N-terminal rat mevalonate kinase IgG preparation) and a polyclonal donkey anti-rabbit IgG conjugated to horseradish peroxidase (1:10,000) as a second antibody. The presence of immune complexes was detected by chemiluminescence using an ECL kit.

Two-dimensional Gel Electrophoresis—The two-dimensional electrophoresis was performed at the University of Michigan protein structure core facility. The solubilized proteins were subjected to isoelectric focusing from pH 3 to 10 using Amersham Biosciences gel strips. The second dimensional electrophoresis was performed using precast 4–12% gradient SDS-polyacrylamide gels. The gels were silver-stained for the detection of proteins.

RESULTS

Purification of LRBP from Rat Ovary—The rat ovarian cytosolic protein LRBP was initially partially purified using a strong cation exchange column as described before (25). Equivalent quantities of partially purified LRBP and total ovarian cytosolic protein (S100 fraction) were separated on a 10% SDS-PAGE and electroblotted onto nitrocellulose. Northwestern analysis was then performed using 32P-labeled LBS of LHR mRNA (25) as described under “Materials and Methods.” The Northwestern blot showed a single band (40 kDa) for both S100 and partially purified LRBP as shown in Fig. 1a. The intensity of the band produced by the partially purified LRBP was stronger than the band produced by crude S100 preparation, indicating that enrichment of LRBP was achieved upon purification through the cation exchange column. The protein(s) that yielded a band in the Northwestern analysis was subsequently eluted from the SDS-polyacrylamide gel and re-natured as described under “Materials and Methods.” The eluted protein(s) was then tested for LHR mRNA binding activity by REMSA using 32P-labeled LBS. The eluted protein(s) exhibited LHR mRNA binding activity as shown in Fig. 1b. In order to examine the homogeneity of the extracted protein(s), an aliquot was subjected to one-dimensional and two-dimensional gel electrophoresis. The Coomassie Blue-stained one-dimensional SDS-PAGE showed only a single band as shown in Fig. 2, and the silver-stained two-dimensional gel showed two spots (Fig. 3) migrating very close to each other, suggesting that both spots most likely represent the same protein. The eluted protein(s) was then subjected to N-terminal sequence analysis for the first 10 amino acids. The resulting sequence (NH2-MLSEVLLVSA-COOH) was then used for a homology search in the Swiss protein sequence data bank. The amino-terminal analysis was performed with purified protein from three separate purifications, and they repeatedly yielded the same sequence data. The homology search revealed the LHR mRNA-binding protein identity to be rat mevalonate kinase (accession number Q03426) (32).

Evidence Supporting the Identity of LHR mRNA-binding Protein as Mevalonate Kinase—Western blot analysis was performed with partially purified LRBP and the SDS-PAGE-separated, gel-extracted LHR mRNA-binding protein(s) fraction (this preparation will be described as “gel-extracted protein(s)” in the rest of the paper) using rat mevalonate kinase antibody as described under “Materials and Methods.” As shown in Fig. 4a, the antibody detected both the partially purified LRBP and gel-extracted protein(s), indicating that the LHR mRNA-binding protein(s) extracted from the gel was indeed mevalonate kinase. Since the two-dimensional electrophoresis of the gel-extracted protein(s) showed two spots (Fig. 3), further analysis was carried out to determine the identity of these spots by Western blot analysis. The result is shown in Fig. 4b. The mevalonate kinase antibody reacted with both spots on the two-dimensional gel, thus establishing the identity of both spots to be mevalonate kinase.

In order to establish that the protein that binds to LHR mRNA in the partially purified LRBP preparation was mevalonate kinase, gel retardation assays were performed with the addition of rat mevalonate kinase antibody as described under “Materials and Methods.” As shown in Fig. 5, inclusion of the
antibody in the binding reaction abolished the RNP complex formation, further establishing the identity of LRBP as mevalonate kinase. RNP complex formation was not abolished when mevalonate kinase antibody preincubated with partially purified LRBP for 30 min at room temperature was added to the binding reaction (Fig. 5b). The purpose of preincubating the mevalonate kinase antibody with partially purified LRBP prior to inclusion in the binding reaction was to block the mevalonate kinase binding sites on the antibody. Similarly, a gel retardation assay performed with an irrelevant antibody (antibody against HBP) also did not cause a decrease in RNP complex formation (Fig. 5b). To further establish the identity of LRBP as mevalonate kinase, a partially purified LRBP preparation was immunodepleted of mevalonate kinase with its antibody using agarose-protein A/G beads as described under “Materials and Methods.” This mevalonate kinase-depleted LRBP preparation was then used for the REMSA. As shown in Fig. 6, a and b, the mevalonate kinase-depleted preparation showed no detectable LHR mRNA binding activity when compared with the LRBP preparation treated with an equal concentration of an irrelevant antibody (HBP antibody) or agarose-protein A/G beads alone. This observation further confirmed the identity of LRBP as mevalonate kinase. Furthermore, this experiment also rules out the possibility that the observed abolishment of RNP complex formation by mevalonate kinase antibody was not due to a direct interaction of the antibody with LHR mRNA.

**Recombinant Rat Mevalonate Kinase and Its Characterization as LHR mRNA-binding Protein**—Rat mevalonate kinase cDNA, generated by RT-PCR using RNA extracted from ovaries of rats that had been treated with hCG, which down-regulates LHR mRNA and up-regulates LRBP, was cloned into pCMV4 expression vector (pCMV4-rMVK) as described under “Materials and Methods.” Human embryonic kidney cells (293 cells) were transiently transfected with pCMV4-rMVK, and cytoplasmic proteins (S100) were prepared after 24- and 48-h transfection. RNA electrophoretic mobility shift analysis was then performed with 32P-LBS using the S100 preparations. As shown in Fig. 7a, the S100 fractions prepared from the mevalonate ki-
nase-transfected cells showed increased LHR mRNA binding activity when compared with S100 fractions prepared from the mock (vector alone)-transfected cells. Western blot analysis performed with the same S100 fractions prepared from 24- and 48-h transfected cells confirmed the overexpression of mevalonate kinase in the pCMV4-rMVK-transfected 293 cells (Fig. 7b). Mevalonate kinase was also detected in mock-transfected cells but at levels lower than in pCMV-rMVK-transfected cells (Fig. 7b, lanes M24 and M48).

To examine the specificity of binding of the overexpressed mevalonate kinase for LHR mRNA, gel shift analysis was performed by incubating the S100 fraction prepared from mevalonate kinase-expressing 293 cells with 32P-LBS in the presence of increasing concentrations of unlabeled LBS (molar excess) and full-length LHR mRNA (molar and mass excess). As shown in Fig. 8a, both unlabeled LBS and full-length LHR mRNA effectively competed for binding to overexpressed mevalonate kinase, in a manner similar to that seen with the purified LRBP (9, 25). The gel shift analysis with increasing concentrations of a different mRNA, /H9252-actin (antisense), did not show competition (Fig. 8b), further supporting the specificity of mevalonate kinase to bind LHR mRNA.

Previous studies with partially purified LRBP have indicated that all cytidine residues in the polypyrimidine-rich bipartite sequence of LBS are necessary for the binding of LRBP (25). We therefore examined whether the recombinant mevalonate kinase also showed requirements for all of the cytidines in the LBS for binding by performing gel shift analysis with increasing concentrations of a mutant LBS in which the cytidines were mutated to uridines. As shown in Fig. 8b, the

Fig. 5. a, RNA mobility shift analysis in the presence of mevalonate kinase antibody. Gel mobility shift analysis was performed with 32P-LBS using partially purified LRBP (2 μg) as described under “Materials and Methods.” Mevalonate kinase antibody (2 and 5 μl) was added to the binding reaction after the RNP complex formation and incubated further at room temperature for 30 min (lanes 3 and 4). Binding reactions were also performed without protein (lane 1) and without mevalonate kinase antibody (lane 2). b, RNA mobility shift analysis using LBS in the presence of mevalonate kinase and HBP antibodies. Gel shift analysis was performed with 32P-LBS using partially purified LRBP (2 μg) as described under “Materials and Methods” (lane 1). Antibodies (2 μl) for mevalonate kinase (lane 2) and HBP (lane 4) were added to the binding reaction after the RNP complex formation and incubated for 30 min at room temperature. Antibodies (2 μl) preincubated with partially purified LRBP (2 μg) were added to the binding reactions (lanes 3 and 5) as negative controls.

Fig. 6. Mevalonate kinase-depleted LRBP preparation shows decreased binding to LBS. RNA mobility shift analysis was performed with 32P-LBS using 2 μg of partially purified LRBP preparation that had been depleted of mevalonate kinase (Fig. 6a, lane 3 and 4) as described under “Materials and Methods.” A control reaction was performed with partially purified LRBP not treated with mevalonate kinase antibody (Fig. 6a, lanes 1 and 2). b, 15 μg of partially purified LRBP was treated with equal concentrations of mevalonate kinase antibody or HBP antibody as a negative control. After 2 h, 15 μl of protein A/G beads were added, and incubation was continued for another 2 h. The reaction mixtures were centrifuged, and the supernatants were collected. Equal aliquots (8 μl) of immunodepleted preparations were then used for the REMSA.
mutant LBS did not compete for mevalonate kinase binding, indicating the importance of cytidines in the LBS for the binding of recombinant mevalonate kinase in a manner similar to that seen with partially purified LRBP preparation from rat ovary (25).

Previous studies have shown that mevalonate kinase, an enzyme involved in cholesterol biosynthesis, binds to both ATP and mevalonate for its catalytic function (33). We therefore examined whether the binding of ATP and mevalonate has any effect on its binding to LHR mRNA. LHR mRNA binding to recombinant mevalonate kinase was tested by REMSA in the presence of increasing concentrations of ATP, UTP, and mevalonate (0.05, 0.5, and 1.0 mM). The results showed that the addition of ATP and mevalonate (Fig. 9, a and b) caused a concentration-dependent decrease in binding of mevalonate kinase to LBS. A complete inhibition of binding of mevalonate kinase to LHR mRNA was seen at 1.0 mM ATP. The inhibitory effect of ATP was specific, since similar inhibition was not seen with UTP even at a 1.0 mM concentration (Fig. 9c). Whereas mevalonate alone caused an inhibition, the effect was less pronounced when compared with ATP. However, the inhibitory effect was substantially increased when 0.05 mM ATP and 0.05 mM mevalonate were added together to the binding reaction when compared with that seen with either 0.05 mM ATP or mevalonate alone.

Since mevalonate caused an inhibition of LHR mRNA binding to mevalonate kinase, further studies were carried out using S100 extracts from cells pretreated with mevastatin, an inhibitor of hydroxymethylglutaryl-CoA reductase to deplete endogenous levels of mevalonate (34, 35). 293 cells were treated with 5 μM mevastatin for 24 h, and gel mobility shift analysis was performed with S100 fractions prepared from mevastatin-and vehicle-treated cells. The results showed that there was an increase in LHR mRNA binding activity in the S100 fraction prepared from mevastatin-treated cells compared with the S100 fraction prepared from control cells (Fig. 9c). These results strongly suggest the possibility that mevalonate kinase acts as an LHR mRNA-binding protein when cholesterol synthesis is down-regulated.

**DISCUSSION**

LH receptor expression during follicular development, ovulation, and luteinization is highly regulated (5, 6). The differences in LH receptor expression are associated with concomitant changes in the steady state levels of LH receptor mRNA (5, 6, 8, 9, 36, 37). Our previous studies have shown that LRBP might be a trans-acting factor in regulating LH receptor mRNA levels in the ovary (8, 9, 25, 26). Therefore, to further characterize this protein as a cytoplasmic trans-acting factor of LHR mRNA regulation, LRBP was purified and identified. The present study describes the purification, identification, and characterization of rat ovarian LRBP. LRBP was initially partially purified from ovarian S100 fraction using cation exchange chromatography, resulting in a 20-fold enrichment (25). The next level of purification was based on the specificity of LRBP for LHR mRNA. The enrichment of LRBP in partially purified LRBP preparation, which was tested previously by performing REMSA (25), was further confirmed by Northwestern analysis (Fig. 1a), showing an intense band by partially purified LRBP preparation when compared with equal amount of S100 fraction. A single prominent band on the Northwestern blot also indicates that there was only one protein binding to the LBS of LHR mRNA. The purification was facilitated by the renaturability of LRBP. We were able to renature LRBP following SDS-PAGE by removing SDS from the protein (Fig. 1b). This renaturability of LRBP has also been seen with other RNA-binding proteins such as iron-responsive element-binding protein (38), c-myc coding region-binding protein (CRD-BP) (24), and yeast poly(A)-binding protein (40). Although two-dimensional electrophoresis performed with the gel-extracted LRBP to test the purity of isolated protein(s) exhibited two spots (Fig. 3) migrating close to each other, Western blot analysis proved that both spots represented MVK (Fig. 4a). The absence of RNP complex formation in the REMSA performed with mevalonate kinase-depleted, partially purified LRBP (Fig. 6, a and b) provides a second line of evidence that LRBP is indeed mevalonate kinase.

The presence of MVK antibody in gel retardation assays performed with partially purified LRBP caused a decrease in RNP complex formation. When an irrelevant antibody (HBP antibody) or MVK antibody preincubated with partially purified LRBP was used, RNP complex formation was not impaired (Fig. 5b). This further supports the idea that the protein interacting with LHR mRNA in partially purified LRBP is MVK. The antibody was raised against the N terminus of MVK (27). Therefore, the decrease in RNP complex formation observed in the presence of MVK antibody in the binding reaction could be due to the blocking of N terminus of MVK by the antibody, which might be involved in LHR mRNA binding. It is also possible that binding of antibody to MVK causes a change in the conformation of MVK, which results in the reduction of its LHR mRNA binding activity.

The direct interaction of rat MVK with rat LHR mRNA was further confirmed by the increased RNP complex formation using the S100 fraction from MVK overexpressed 293 cells (Fig. 7, a and b). The competition studies performed with unlabeled full-length LHR mRNA and 40-nucleotide LBS (Fig. 8a) clearly indicate that the overexpressed rat MVK specifically binds to LHR mRNA and that the coding region between nucleotides 188 and 228 of LHR mRNA interacts with the recombinant MVK. The competition studies performed with unlabeled mutant LBS (all cytidines required for the LRBP binding in wild type LBS were mutated to uridines) and β-actin antisense RNA (Fig. 8b) demonstrate that cytidine residues in the LBS are...
important for the binding of recombinant MVK and that the binding is specific to LHR mRNA.

Mevalonate kinase is a member of GHMP kinase superfamily, a family of catalytic enzymes with a highly conserved glycine-rich motif (PXGXGLGSSAA) that is implicated in the binding of ATP (41–43). It might first appear that the presence of an ATP binding site in MVK might make it vulnerable for nonspecific binding to any RNA molecule due to the presence of adenine residues. This possibility, however, was ruled out on the basis of competition studies performed with mutant LBS. The inability of mutant LBS (in which all C residues were mutated to U) to compete with labeled wild type LBS to bind to LHR mRNA clearly shows that the interaction of LHR mRNA with mevalonate kinase is independent of adenine residues. This is corroborated by findings here, competition of substrates or cofactors for RNA binding has been reported with other enzymes such as thymidylate synthase and dihydrofolate reductase (44). This β-α-β loop has been found to be the primary site of S5 and S9 30 S ribosomal proteins for interacting with RNA backbone of 30 S ribosomal subunit (45). Interestingly, a new member of this GHMP family, XOL-1, has been shown to be a mediator of sexual differentiation in C. elegans (46). Other catalytic proteins have also been reported as RNA-binding proteins (47). The well characterized iron regulatory protein, which is required for iron homeostasis in cells, is the cytosolic enzyme aconitase (12, 48). The enzymes thymidylate synthase and dihydrofolate reductase bind to their own mRNAs and repress translation (15, 16). The two glycolytic enzymes, glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase, bind to AU-rich elements in the 3′-untranslated regions of mRNAs (49, 50). Their role in AU-rich element-mediated mRNA regulation remains to be elucidated. Lactate dehydrogenase has been shown to be directly interacting with AUF1, a protein implicated in AU-rich element-mediated mRNA regulation (50). Consistent with our findings here, competition of substrates or cofactors for RNA binding has been reported with other enzymes such as thymi-
dylate synthase, dihydrofoltate reductase, glyceraldehyde-3-phosphate dehydrogenase, and lactate dehydrogenase (15, 16).

Mevalonate kinase is an enzyme involved in de novo synthesis of cholesterol. In the ovary, the interaction of LH with LH receptor is a key event in the regulation of synthesis of steroid hormones from cholesterol (1). It has been well established that LH receptor expression undergoes down-regulation when exposed to high levels of LH/hCG. This down-regulation of LH receptor is secondary to full activation of cellular steroidogenic enzymes. In the ovary, the interaction of LH with LH receptor expression. This type of regulation appears to be mediated by switching function to meet different metabolic needs by new members. The multifunction of proteins may be beneficial for cells by switching function to meet different metabolic needs.

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