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Molecular Regulation of Gonadotropin Receptor Expression: Relationship to Sterol Metabolism*

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Abstract

We have identified a specific LHR mRNA binding protein that selectively binds to the polypyrimidine rich bipartite sequence in the coding region of the LHR mRNA and accelerates its degradation. This process has been shown to be one of the mechanisms that is responsible for the loss of the steady state levels of LHR mRNA following the preovulatory LH surge or the down regulation of the receptor in response to the administration of a pharmacological dose of LH or hCG. The *trans* factor, designated as the LHR mRNA binding protein (LRBP), was purified and its identity was established as being mevalonate kinase, an enzyme involved in cholesterol biosynthesis. When mevalonate kinase expression was abolished by treating cultured luteal cells with 25-hydroxycholesterol, the ability to undergo LH-induced down regulation of LHR mRNA was completely abrogated. Examination of the crystal structure of mevalonate kinase coupled with mutagenesis of the critical residues in the catalytic site revealed that the catalytic site is in close proximity to the LHR mRNA binding site. Further studies revealed that mevalonate kinase causes LHR mRNA degradation by acting as a translational suppressor by forming an untranslatable ribonucleoprotein (RNP) complex which is then targeted for degradation. These studies show that LHR expression in the ovary is regulated by a post-transcriptional mechanism mediated by mevalonate kinase thereby linking LHR expression with cholesterol metabolism.

Keywords

Leutinizing hormone receptor; mevalonate kinase; post transcriptional regulation; mRNA-binding protein; Human Chorionic gonadotrophin

1. Introduction

The LH/hCG receptor (LHR) is expressed primarily in the gonads although its expression in non-gonadal tissues has now been well-established (Ascoli et al., 2002; Menon et al., 2004; Rao, 2001). LHR belongs to the family of rhodopsin/β2 adrenergic receptor subfamily of G protein coupled receptors (McFarland et al., 1989). Other members of the glycoprotein hormone receptor family include Follicle Stimulating Hormone (FSH) receptor and Thyroid Stimulating Hormone (TSH) receptor. Upon binding of the LH or hCG to the extracellular domain, the receptor undergoes a conformational change. This leads to the GDP-GTP

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exchange on the cognate G protein resulting in the dissociation of the inhibitory beta-gamma subunit from the alpha subunit leading to the activation of adenylate cyclase. This results in an increase in the intracellular concentration of adenosine 3′, 5′ cyclic monophosphate (cAMP). Cyclic AMP then activates protein kinase cascade and activates multiple signaling pathways culminating in increased steroidogenesis and stimulation of other cellular functions. At higher concentrations, LH or hCG can also activate phospholipase C resulting in the formation of inositol triphosphate (IP_3) and diacylglycerol (DAG) (Davis et al., 1984; Fu et al., 2002; Gudermann et al., 1992; Munshi et al., 2001).

In the ovary LHR is expressed in the follicles, theca cells and corpus luteum. The expression of LHR shows considerable changes during the ovarian cycle (Hoffman et al., 1991; LaPolt et al., 1990; Segaloff et al., 1990). The growing follicles acquire LHR by the combined actions of FSH and estradiol (Zeleznik, 2004). In response to pre-ovulatory surge, the receptor expression is transiently down regulated followed by a full recovery and reaching maximum level by the mid luteal phase (Hoffman et al., 1991; LaPolt et al., 1990; Peegel et al., 1994; Segaloff et al., 1990). The receptor level then falls off with the regression of the corpus luteum. The mechanism by which LH or hCG regulates LHR expression has been a topic of interest in our laboratory. Using rat and human ovarian models, we have shown that LHR expression is regulated through post-transcriptional mechanisms (Lu et al., 1993; Nair et al., 2006). A specific LHR mRNA binding protein has been shown to be responsible for this process. The LHR mRNA binding protein was then identified as mevalonate kinase (MVK), an enzyme involved in cholesterol biosynthesis. This article will focus on the discovery of this phenomenon, the characterization of the LHR mRNA binding protein and some insight into the mechanism by which this RNA binding protein regulates LHR mRNA expression.

2. LHR down regulation

The down regulation of LHR occurs in response to preovulatory LH surge or in response to the administration of a pharmacological dose of LH or hCG. The loss of the ligand binding activity is closely coupled to the loss of the steady state levels of LHR mRNA. In human, this phenomenon occurs during ovulation induction by the administration of hCG prior to ovum retrieval for *in vitro* fertilization (Nair et al., 2006). Granulosa cells isolated from the retrieval fluids showed complete loss of LHR mRNA on the day of retrieval, when examined by Northern blot analysis (fig 1) (Nair et al., 2006). The granulosa cells were then cultured for different periods of time and RNA was extracted from the harvested cells for examination of the reappearance of LHR mRNA. The recovery from down regulation began approximately 48 hours later and reached maximum level by day 4 in culture (fig 1). This phenomenon can be demonstrated in a pseudopregnant rat model by treatment with a single dose of 50 IU of hCG to down regulate LHR. The loss of LHR mRNA transcripts during down regulation is shown in fig 2A. The left hand panel shows the expression of LHR mRNA transcripts in the saline treated control ovaries. The expression of LHR mRNA transcripts in response to the administration of hCG that mimics preovulatory LH surge is shown on the right hand panel. The LHR mRNA expression remains suppressed up to 48 hours and recovers from down regulation by 72 hours (Hoffman et al., 1991; Lu et al., 1993). The loss of receptor mRNA could be either due to a decrease in the rate of synthesis or due to increased degradation. To examine these possibilities, nuclear run-on assays were performed to determine the transcription rate during hCG-induced LHR down regulation (Lu et al., 1993). The results showed that the transcription rate remained identical in both control and hCG treated groups suggesting that the loss of the steady state levels of LHR mRNA is not due to decreased transcription, but rather resulted from increased degradation (Lu et al., 1993). Furthermore, the half- life of LHR mRNA was significantly reduced in the hCG treated group when compared to the mRNA decay rate of the control group (fig 2B) (Lu et

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al., 1993). There was approximately a threefold decrease in mRNA half- life in the down regulated group.

The steady state level of mRNA, in general, is controlled by the rate of its synthesis and the rate of degradation. It is now clear that highly regulated mRNAs are controlled, at least in part, by modulating their degradation. In the majority of instances of post-transcriptional regulation of mRNA, the changes in the stability of mRNA appear to result from changes in the binding of cytoplasmic proteins, known as *trans* factors, to defined sequences or structures, known as *cis* elements, in the target mRNA, forming a ribonucleoprotein complex. These *trans* factors can either increase or decrease the stability of RNA. Some RNA binding proteins recognize sequences in the coding region of the mRNA as is the case with c-fos, c-myc, thymidylate synthase and dihydrofolate reductase (Bernstein et al., 1992; Lin et al., 2000; Shyu et al., 1991; Tai et al., 2004). Others can bind to the 5′-untranslated region as demonstrated by the binding of iron response element binding protein to ferritin mRNA involved to regulate iron homeostasis (Leibold and Munro, 1988). In other instances, RNA binding proteins have been known to interact with the 3′UTR as in the case with beta adrenergic receptor mRNA and Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) mRNA (Iwai et al., 1991; Tholanikunnel and Malbon, 1997). Thus, on the basis of these previous studies, the possible existence of an LHR mRNA binding protein was examined in the cytosolic fractions from the ovaries after injection with a dose of hCG that is known to down regulate LHR mRNA expression.

3. Identification of LHR mRNA binding protein

Since our studies suggested that LHR mRNA expression is post-transcriptionally regulated, our first attempt was to examine if this post-transcriptional mechanism involves the participation of an LHR mRNA binding protein. To examine this, we prepared cytosolic fractions from control and down regulated ovaries and the ability of these fractions to bind LHR mRNA was examined (Kash and Menon, 1998). This was accomplished by performing RNA electrophoretic mobility shift assay (REMSA). Briefly, a 100,000 × g supernatant (S100) fraction of the ovarian homogenate was incubated with [^{32}P] labeled LHR mRNA that was prepared by transcribing the full length cDNA encoding the LHR in the presence of [α^{32}P] UTP. After treatment with RNAses to degrade unreacted [^{32}P] labeled RNA, the ribonucleoprotein complex was subjected to electrophoresis under non-denaturing conditions, and autoradiography. Using this technique, we compared the ability of the S100 fractions from the control and hCG down regulated ovaries to bind radiolabeled LHR mRNA. The results showed that there were two prominent bands designated as LRBP-1 and LRBP-2, corresponding to MW=50 kDa and 45 kDa, respectively, in both the control and down regulated ovaries (Kash and Menon, 1998). Furthermore, the intensity of LRBP-1 was about 3 fold higher in the down-regulated group compared to the saline treated control, with no significant change in the intensity in LRBP-2. Since the hormonal inducibility of LRBP-1 was significantly higher, in future studies we focused our attention on LRBP-1.

Extensive studies were then carried out to determine the nucleotide sequence involved in the interaction of LHR mRNA with the proteins in the S100 fraction. Truncated segments of the full length LHR mRNA were incubated with ovarian S-100 fractions and electrophoretic mobility shift assays were performed to localize the region of LHR mRNA that interacted with the protein. The results of these studies coupled with the results from RNA hydroxyl radical foot- printing revealed that the contact site of the interaction of LRBP-1 with LHR mRNA consisted of a bipartite polypyrimidine- rich sequence corresponding to the nucleotides 203–220 located in the region encoding to the amino terminus of LHR (Kash and Menon, 1999).

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After establishing the existence of an inducible RNA binding protein that recognizes a specific structure of the LHR mRNA, we examined the LHR mRNA binding activity under conditions that mimic follicle maturation and the LH surge (Nair et al., 2002). Since the expression of the LHR is increased during follicle maturation and, conversely, the LHR expression is decreased during down regulation, these two paradigms were selected to examine whether changes in LHR expression have any bearing on the LHR mRNA binding activity. In response to treatment of 23 day old rats with PMSG, there was an increase in the expression of LHR mRNA by 56 hours, as expected, due to the induction of LHR mRNA by the FSH type of activity associated with PMSG. Fifty six hours after PMSG treatment, administration of 50 IU of hCG caused a transient decrease in LHR mRNA expression during the ensuing 24 hour period as a result of down regulation. The LHR mRNA level returned to the hCG pretreatment level by 72 hours. Thus, the changes in LHR mRNA were consistent with the expected up and down regulation following the treatments. The LHR mRNA binding activity showed a strikingly different pattern in response to these treatments. When the expression of LHR mRNA was high in response to PMSG treatment, the LRBP activity showed a decrease. Conversely, an increased expression of LHR mRNA binding activity was seen when LHR mRNA expression was low in response to LHR down regulation (Nair et al., 2002). Thus, there appeared to be an inverse relationship between the expression of LHR mRNA binding activity and LHR mRNA expression. These results clearly pointed out that LRBP is a regulator of LHR mRNA expression.

4. Characterization of LHR mRNA binding protein

We then proceeded to determine the identity of the LHR mRNA binding protein. Initial approaches for the purification of LRBP focused on standard biochemical techniques. Although cation exchange chromatography yielded some degree of purification, further purification was not successful using RNA affinity column since the elution of the RNA binding protein from the matrix turned out to be a daunting task. We succeeded in purifying this protein to homogeneity by a combination of cation exchange chromatography followed by electrophoresis. After partial purification of the ovarian S-100 fractions on cation exchange columns, the concentrated samples were subjected to SDS-PAGE. After transfer of the gel to nitrocellulose membrane, it was overlaid with a buffer containing [^{32}P] labeled LHR mRNA binding sequence consisting of a 40-mer of LHR mRNA. Autoradiography revealed a single band that interacted with the LHR mRNA. A corresponding band from an SDS-PAGE performed in an identical manner was cut and its purity was confirmed by subjecting the eluted protein to one-dimensional and two-dimensional gel electrophoresis. The protein appeared as a homogeneous single band (Nair and Menon, 2004). The purified protein band was eluted from the gel and subjected to both N-terminal analysis and determination of partial sequences using MALDI-TOF.

The results from amino terminal analysis and MALDI-TOF revealed the identity of the protein as being mevalonate kinase (Nair and Menon, 2004). The identity of the protein was further established by immunoblotting the partially purified and the gel purified protein with anti mevalonate kinase antibody (Nair and Menon, 2004) and by electrophoretic mobility shift assay to determine its ability to bind LHR mRNA. Since rat MVK had been cloned previously (Tanaka et al., 1990), we were able to clone the cDNA encoding rat mevalonate kinase based on the published sequence information (Nair and Menon, 2004). The protein was then over-expressed in 293T cells and the overexpressed protein was subjected to western blotting with anti mevalonate kinase antibody. The LHR mRNA binding activity of the expressed protein was then determined by performing RNA electrophoretic mobility shift assay. The results from the studies using overexpressed protein were in agreement to that seen using S-100 fractions and the gel purified protein (Nair and Menon, 2004).

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Mevalonate kinase is an enzyme involved in cholesterol biosynthesis. It converts mevalonate to 5-phospho mevalonate, a precursor of isoprene units for the synthesis of FPP, squalene and cholesterol. Although this finding was unexpected, a review of the literature revealed that several metabolic enzymes have now been identified with RNA binding properties with defined regulatory functions in RNA metabolism (Ciesla, 2006).

5. Evidence for mevalonate kinase as a regulator of LHR mRNA expression

The results presented so far show a close relationship between LHR mRNA expression and LHR mRNA binding activity, and that the LHR mRNA binding protein might act as a *trans* factor in regulating LHR mRNA expression. The next goal was to show a direct role of mevalonate kinase in the expression of LHR mRNA in the ovarian tissue. Attempts were made to block mevalonate kinase expression in primary cultures of human luteinized granulosa cells using siRNA with little success, primarily due to the inability to transfect primary cultures with siRNA with reasonable efficiency. However, metabolic regulation of mevalonate kinase, an enzyme encoded by a gene containing an oxysterol response element, has been described by Brown and Goldstein (Adams et al., 2004). The genes encoding key enzymes in the cholesterol biosynthetic pathway contain sterol response element (SRE) in the promoter region, and the expression of these enzymes are regulated by cholesterol and 25-hydroxycholesterol. High cholesterol and 25-hydroxycholesterol have been shown to suppress the expression of several key enzymes involved in cholesterol biosynthesis. We used this approach to block the synthesis of mevalonate kinase by treating human luteinized granulosa cells with 25-hydroxycholesterol.

The SRE containing genes are regulated by sterol response element binding factor (SREBF) previously called serum response element binding protein (SREBP) (Brown and Goldstein, 1997). SREBF is localized in the endoplasmic reticulum in a precursor form that is bound to another protein, SEBF cleavage activating protein (SCAP) which serves as a chaperone. The SREBF-SCAP complex is bound to an ER membrane protein, Insig. When the cellular cholesterol concentration is low, SCAP-SREBF precursor complex is transported from the ER to Golgi via COP 2 vesicles and the SREBF precursor is proteolytically processed in the Golgi. The active form of SREBF is then transported to the nuclei and binds to SRE of the genes coding for cholesterol biosynthetic enzymes and other proteins involved in cholesterol homeostasis such as LDL receptor. Activation of SRE promotes the expression of cholesterol biosynthetic enzymes. Conversely, when the cholesterol supply is abundant, the SREBF-SCAP complex is retained in the ER. Consequently, the expression of SRE-containing genes and the enzymes encoded by the cognate genes, including mevalonate kinase, is suppressed. We used this strategy to suppress mevalonate kinase expression by treating cultured human granulosa cells collected from the IVF retrieval fluids with 25-hydroxycholesterol. A second group of cultures were treated with hCG to down regulate LHR mRNA. A third group of cultures were pretreated with 25-hydroxycholesterol followed by hCG. The results presented in fig 3 show that treatment with hCG alone increased the expression of mevalonate kinase mRNA, but inclusion of 25-hydroxycholesterol, as expected, inhibited the expression of mevalonate kinase mRNA. This is predicted on the basis of the suppressive effect of 25-hydroxycholesterol on mevalonate kinase expression. Most importantly, while hCG treatment alone caused down regulation of LHR mRNA expression, inclusion of 25-hydroxycholesterol along with hCG prevented the down regulation of LHR mRNA (fig 4). Thus, suppression of mevalonate kinase expression abolished hCG-induced down regulation of LHR mRNA. This experiment provides direct experimental proof demonstrating that mevalonate kinase is a *trans* factor involved in LHR mRNA expression. Our results are supported by the findings of Ikeda et al who showed that the induction of LHR in cultured rat granulosa cells by FSH and estradiol was abrogated by the overexpression of mevalonate kinase (Ikeda et al., 2008).

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6. Mechanism of mevalonate kinase-mediated regulation of LHR mRNA expression

Recent studies have revealed the participation of the active site of other metabolic enzymes that serve as RNA binding proteins. Structural studies of cytosolic form of aconitase, identified as iron regulatory protein 1 (IRP1) which binds iron regulatory elements present in the 3′ untranslated regions of the mRNAs, showed extensive overlap between the catalytic center and its RNA binding site (Dupuy et al., 2006; Klausner et al., 1993). The catalytic mechanism of mevalonate kinase has been well worked out using information derived from crystal structure as well as by mutagenesis (Cho et al., 2001; Fu et al., 2002). As stated earlier, mevalonate kinase catalyzes the phosphorylation of mevalonate to form phophomevalonate. According to the proposed mechanism, the active site of mevalonate kinase is composed of Asp, Lys, Glu and Ser residues. Lys binds ATP and the transition state of Mg^{2+}-ATP complex is stabilized by Glu and by Ser. Asp acts as a general base catalyst abstracting a proton from mevalonate followed by the transfer of the phosphate from ATP to mevalonate to form phosphomevalonate (Fu et al., 2002). To determine the proximity of the catalytic center to the LHR mRNA binding site of mevalonate kinase, we examined whether the two substrates, ATP and mevalonate, are able to compete with LHR mRNA to bind to mevalonate kinase. The results clearly showed that both substrates effectively competed with LHR mRNA for binding to mevalonate kinase (fig 5) (Nair and Menon, 2004). This suggested that the active site might overlap with the LHR mRNA binding site. Furthermore, mutagenesis of the residues in the active site of the enzyme reduced the ability to bind to LHR mRNA, further supporting the role of the active site of the enzyme in mRNA recognition (Nair et al., 2008).

The site of LHR mRNA binding to mevalonate kinase was then examined using structural information. Mevalonate kinase belongs to a family of enzymes that include galactokinase, homoserine kinase, mevalonate kinase and phosphomevalonate kinase (Zhou et al., 2000). These 4 enzymes contain a motif composed of a left handed, β– α– β fold. This fold is known as ribosomal protein S5 domain 2 like fold, that is found in proteins that interact with nucleic acids, such as RNase P and ribosomal protein S5 and S9 binding to RNA backbone of the 30S ribosomes (Carter et al., 2000). In comparison to the β– α– β fold present in the two ribosomal proteins, we identified the β– α– β fold in rat mevalonate kinase, shown in Cyan (fig 6). Curiously, the base of the β– α– β fold touches the site where ATP binds and this allows interactions with a number of residues in the β– α– β fold. Thus, we propose that the nucleotides in LHR mRNA bind to the region in close proximity to the region that exhibits the β– α– β fold.

Since mRNA decay has been shown to be associated with translation, we tested the effect of mevalonate kinase on the *in vitro* translation of LHR mRNA using a rabbit reticulocyte lysate system (Nair and Menon, 2005). Using this system, the translation of full length LHR mRNA with FLAG sequence attached to the carboxyl terminus was examined by determining the incorporation of [^{35}S] methionine followed by immunoprecipitation of the LHR with anti FLAG antibody. The immunoprecipitated LHR was subjected to electrophoresis and autoradiography. Addition of mevalonate kinase resulted in an inhibition of LHR mRNA translation while a non-specific protein such as BSA had no effect. The suppressive effect of mevalonate kinase on the translation of LHR mRNA was specific, since addition of mevalonate kinase produced no inhibition of the translation of actin mRNA. The inhibitory effect of mevalonate kinase was reversible by adding excess amount of the LHR mRNA binding sequence. This reversal of the inhibitory effect was specific since addition of mutated LHR mRNA binding sequence was ineffective (Nair and Menon, 2005). Thus, we conclude that the binding of LHR mRNA to mevalonate kinase forms an untranslatable ribonucleoprotein complex and targets it to a degradative pathway.

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Since mevalonate kinase does not contain any nucleolytic activity, it is likely that it has to interact with other proteins to cause mRNA degradation. To identify mevalonate kinase interacting proteins, we used a yeast two hybrid screen. These studies are currently in progress.

In summary, our results show that LH receptor expression in the ovary is regulated post-transcriptionally by mevalonate kinase which acts as an RNA binding protein. We propose that during LH/hCG-induced down regulation, steroidogenesis is temporarily stopped. Mevalonate kinase then switches its function from its normal catalytic function to an LHR mRNA binding protein. The binding of mevalonate kinase to LHR mRNA prevents its translation resulting in the degradation of LHR mRNA leading to a loss of cell surface receptor. When steroidogenic function is restored, mevalonate kinase recovers its catalytic function thereby restoring the ability of the cell to express LHR. Thus mevalonate kinase, an enzyme involved in cholesterol biosynthesis, plays a regulatory role in LH receptor mRNA expression in the ovary.

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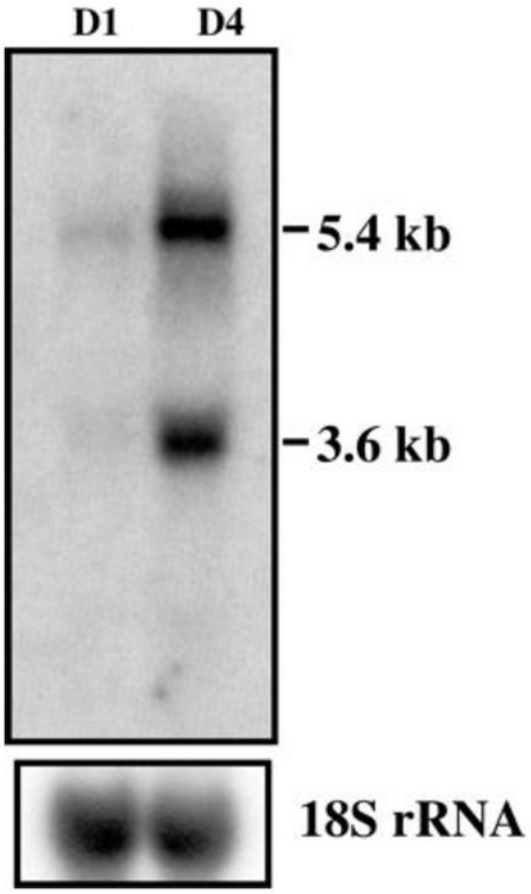

Fig 1.

Northern blot analysis of LHR mRNA in human granulosa cells immediately after retrieval (D1) and after 4 days of incubation in serum free media (D4). Total RNA was extracted from granulosa cells from day 1 and four days of incubation (D4) to recover from downregulation. RNA was separated on agarose-formaldehyde gel, transferred to nitrocellulose membranes, hybridized with the ^{32}P-labeled hLHR cDNA, and exposed to x-ray film. To monitor RNA loading, the blot was stripped and rehybridized with radiolabeled cDNA for 18S rRNA. The blot shown is one representative of three experiments with similar results. *Copyright 2006, The Endocrine Society*.

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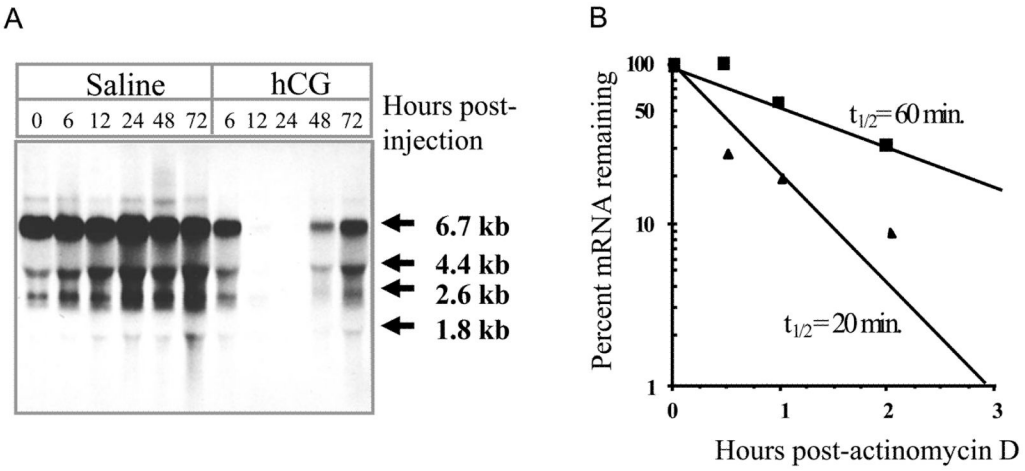

Fig 2.

Hormonal control of LHR mRNA expression in the ovary. A. Northern blot hybridization analysis of steady state LHR mRNA levels during hCG-induced down-regulation. Autoradiogram of Northern blot hybridization analysis of total RNA isolated at the indicated times from the ovaries of saline-injected (control) (*lanes 1–6*) or hCG-injected (down-regulated) (*lanes 7–11*) rats. Blots were probed using a labeled cDNA encoding the LHR carboxyl terminus and a portion of the 3′-UTR (nucleotides 1936–2682). B. LHR mRNA half-life determination in control and 12-h downregulated rat ovaries. Cell suspensions were incubated with 10 μg/ml actinomycin D for 2h. Duplicate aliquots of 20×10^6 cells were removed at the indicated times. Total RNA was isolated and assayed for LHR mRNA by solution hybridization. Each data point represents the average of duplicate determinations. ■ Contol, ▲ downregulated.

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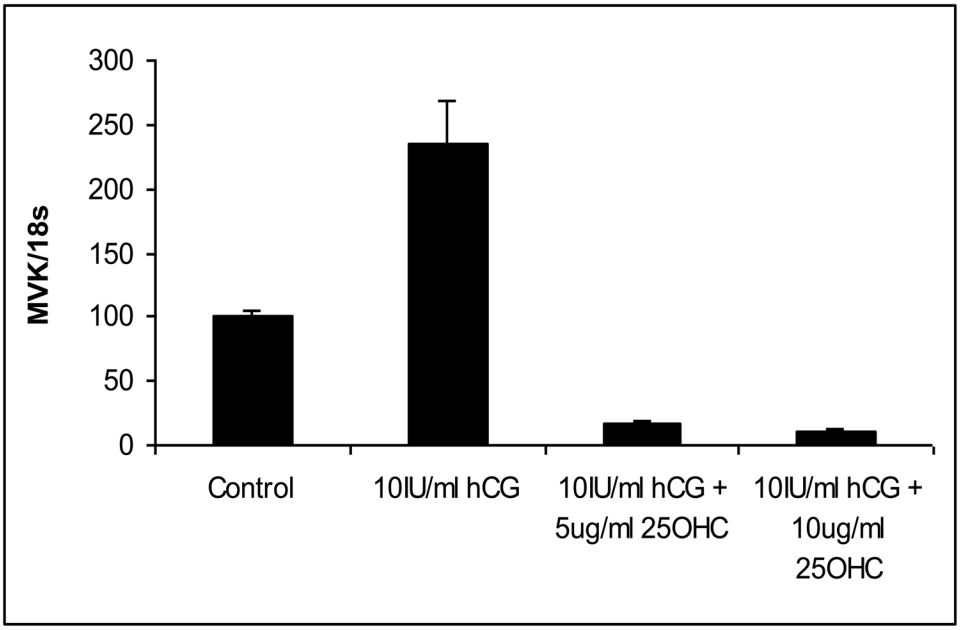

Fig 3.

25-hydroxy cholesterol (25-OHC) inhibits Mvk Gene Expression in Human Granulosa Cells. After culture in serum free medium for 48 h, cells were treated with serum free medium, or 10 IU/ml hCG, or 10 IU/ml hCG plus 10 μg/ml 25-OHC, and harvested at 0 and 12 h. Steady-state levels of Mvk was measured by real-time PCR. Mean values ± SE (n = 3) were normalized to 18S rRNA and graphed as percent of control (time 0 h). *$P < 0.05$.

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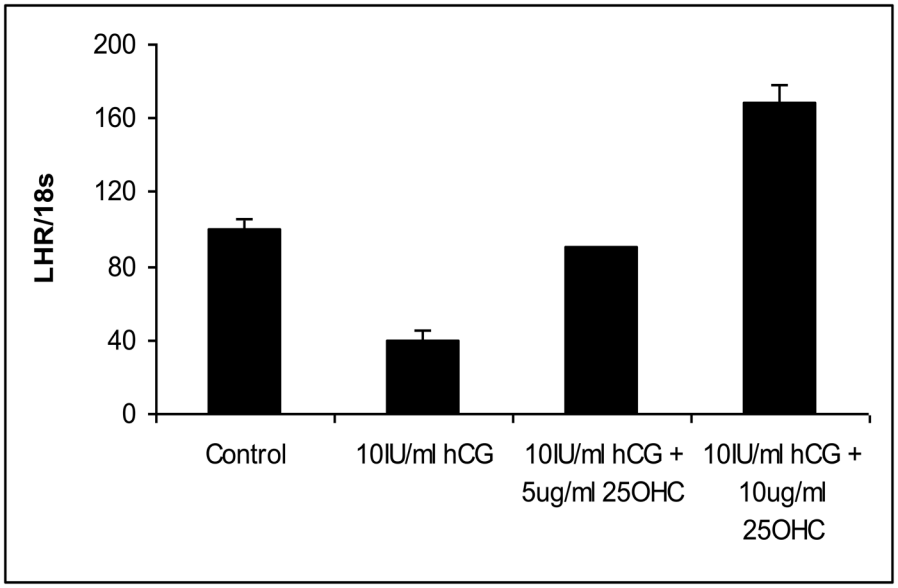

Fig 4.

25-hydroxy cholesterol (25-OHC) abolishes hCG-mediated regulation of LHR mRNA. After culture in serum free medium for 48 h, cells were cultured in serum free medium, in the presence or absence of 10 IU/ml hCG, or 10 IU/ml hCG plus 10 μg/ml 25-OHC, and harvested at 0 and 12 h. Steady-state levels of LHR mRNA was measured by real-time PCR. Mean values ± SE (n = 3) were normalized to 18S rRNA and graphed as percent of control (time 0 h). *$P < 0.05$. *Copyright 2007, The Endocrine Society*.

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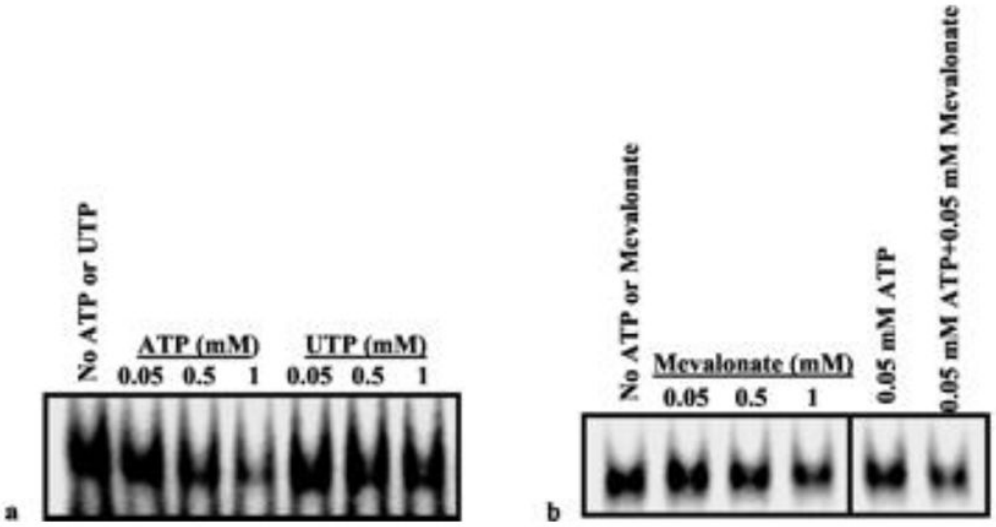

Fig 5.

Lanes *a* and *b*, ATP and mevalonate compete for the binding of recombinant mevalonate kinase to LHR mRNA. RNA gel mobility shift analysis of the binding of ^{32}P-labeled LHR mRNA sequence (LBS) with 10 μg of S100 fraction prepared from 293 cells 48 h after transfection with pCMV4-rMVK was performed (Nair and Menon, 2004). ATP and UTP (magnesium salts) and mevalonate were added to the binding reactions in the concentrations indicated. *Copyright 2004, ASBMB.*

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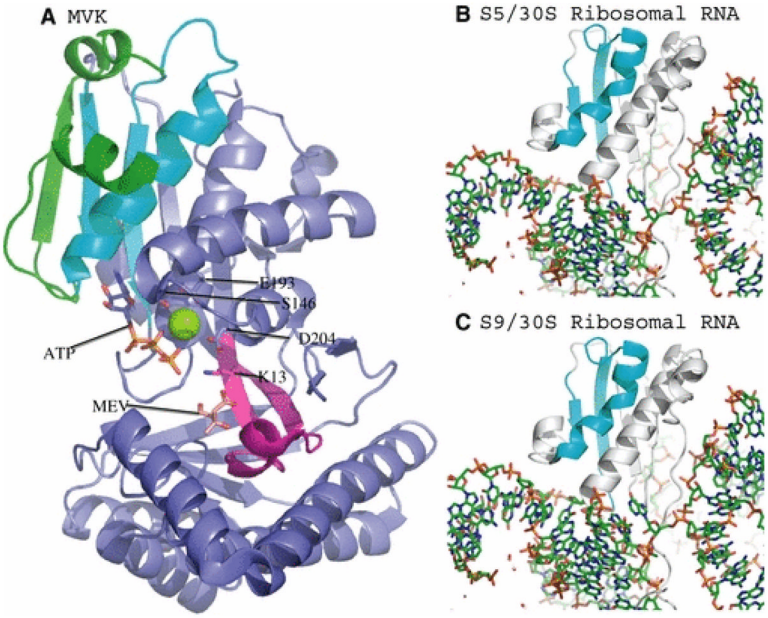

Fig 6.

(A) Structure of rat MVK bound to ATP-Mg^{2+} (Fu et al., 2002) with a model of mevalonate based on the crystal structure of mevalonate bound to *Leishmania major* MVK (Sgraja et al., 2007). Residues comprising the left-handed β–α–β motif are colored cyan. The β–α–β motif is disrupted by a 50 residue insert that is colored green. ATP and mevalonate are shown in stick representation. Residues forming the base of the mevalonate binding pocket, Gly12 to Leu33, are colored magenta. The side chains of Asp204 interacting with Lys33 are indicated, as are the side chains of Glu193 and Ser146 interacting with the Mg^{2+} ion (green sphere). The model was constructed by superimposing the crystal structure of *L. major* MVK bound to mevalonate onto the structure of rat MVK bound to ATP-Mg^{2+}. Mevalonate from the *L. major* structure was then extracted onto the rat MVK complex. (B, C) Structures of two other left-handed β–α–β motifs interacting with RNA are shown for S5 and S9 ribosomal RNA proteins both bound to the 30S ribosome (Carter et al., 2000). *Copyright 2008, Federation of European Biochemical Societies*.