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Regulation of luteinizing hormone receptor mRNA expression by a specific RNA binding protein in the ovary☆

K.M.J. Menon[a,b,*], Anil K. Nair[a,b], Lei Wang[a,b], Helle Peegel[a,b]

[a] *Department of Obstetrics and Gynecology, The University of Michigan Medical School, 6428 Medical Science I, 1150 W. Medical Center Drive, Ann Arbor, MI 48109-0617, United States*

[b] *Department of Biological Chemistry, The University of Michigan Medical School, Ann Arbor, MI 48109-0617, United States*

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Abstract

The expression of LH receptor mRNA shows significant changes during different physiological states of the ovary. Previous studies from our laboratory have identified a post-transcriptional mechanism by which LH receptor mRNA is regulated following preovulatory LH surge or in response to hCG administration. A specific binding protein, identified as mevalonate kinase, binds to the open reading frame of LH receptor mRNA. The protein binding site is localized to nucleotides 203–220 of the LH receptor mRNA and exhibits a high degree of specificity. The expression levels of the protein show an inverse relationship to the LH receptor mRNA levels. The hCG-induced down-regulation of LH receptor mRNA can be mimicked by increasing the intracellular levels of cyclic AMP by a phosphodiesterase inhibitor. An *in vitro* mRNA decay assay showed that addition of the binding protein to the decay system caused accelerated LH receptor mRNA decay. Our results therefore show that LH receptor mRNA expression in the ovary is regulated post-transcriptionally by altering the rate of mRNA degradation by a specific mRNA binding protein.

Keywords: Mevalonate kinase; LHR mRNA; Post-transcriptional regulation

1. Introduction

The ovary is a unique organ, as in most mammals its structure and function undergo continuous change during the reproductive life span. In rodent and human, luteinizing hormone (LH) plays a critical role of stimulating androgen biosynthesis by the theca-interstitial cells during follicular development. The androgens serve as substrate for estrogen biosynthesis by the granulosa cells (Bjersing, 1967). Follicular growth is stimulated mainly by FSH, and possibly augmented by estradiol and insulin/IGF-1 system (Hirshfield, 1991; Zeleznik, 2004). The growing follicles acquire LH receptors by the combined actions of FSH and estradiol. In most mammals, ovulation occurs in response to the preovulatory LH surge when the oocyte reaches the metaphase of the second meiotic division (Baker, 1972). Dramatic changes occur in the follicle following ovulation that involve the transformation of granulosa cells to lutein cells resulting in the formation of corpus luteum within the cortex of the ovary. During this period of transition, a refractory period of LH responsiveness occurs that is characterized by a transient loss of LH receptors and uncoupling of the LH receptor from the cognate G protein-coupled responsive system (Hunzicker-Dunn et al., 1979; Menon et al., 2004). This is followed by the full recovery from down-regulation by increasing the expression of LH receptors, which peaks around the mid-point of the luteal phase to support the steroidogenic function of the ovary in order to make the endometrium receptive for the implantation of the blastocyst. If implantation of the blastocyst does not occur, the LH receptors decline as the corpus luteum undergoes atresia. Thus, the expression of LH receptor shows dramatic changes during the ovarian cycle in response to the changing hormonal milieu, principally the changes in the levels of FSH and LH. While FSH along with other paracrine factors is known to regulate the development of the primary follicles to preantral and antral stages, the LH receptor makes its appearance in signifi-

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* Corresponding author at: Department of Obstetrics and Gynecology, The University of Michigan Medical School, 6428 Medical Science I, 1150 W. Medical Center Drive, Ann Arbor, MI 48109-0617, United States.
Tel.: +1 734 764 8142; fax: +1 734 936 8617.
E-mail address: kmjmenon@umich.edu (K.M.J. Menon).

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cant amounts as a result of FSH stimulation (Menon et al., 2005; Zeleznik, 2004). Since FSH acts through the interaction with its receptors localized on plasma membranes, followed by the activation of adenylate cyclase (Channing et al., 1980; Zeleznik, 2004), it is conceivable that the induction of the LH receptor by FSH involves cyclic AMP responsive transcriptional activation. However, other mechanisms for the induction of LH receptors might also operate in these cells. As the ovary acquires LH receptors (Zeleznik et al., 1974), their expression does not remain at a constant level; instead it changes throughout the ovarian cycle. It should be noted that this occurs, at least in part, due to changes in the circulating levels of LH. In this study, we have focused our attention on the molecular mechanism involved in the changes in LH receptor expression during different physiologic states of the ovary.

2. LH receptor mRNA expression during ligand-induced down-regulation

Previous studies have shown that in the rat, preovulatory LH surge produces a rapid decline in LH receptor expression as well as an uncoupling of LH receptor from the cognate G proteins (Hoffman et al., 1991; Lapolt et al., 1990; Lu et al., 1993). The loss of LH receptor from the cell surface may be explained on the basis of the rapid endocytosis of the LH-receptor complex, causing its depletion. The steady state level of receptor expression is the balance between the rate at which the receptor is trafficked to the cell surface and the rate of its endocytosis. The loss of steady state levels of receptor expression could also be due to a temporary pause in synthesis by decreased transcription, or an increase in degradation of mRNA. To examine these possibilities, we used pseudopregnant rats as a model system. Immature rats were treated with 50 IU PMSG followed by 25 IU hCG 56 h later to induce pseudopregnancy. On day 4 of pseudopregnancy, one set of animals was injected with 50 IU hCG, and the control set received saline. Ovaries were collected at different time intervals up to 72 h (Hoffman et al., 1991; Lu et al., 1993; Peegel et al., 1994) and were processed for Northern blot analysis for the expression of LH receptor mRNA, for *in situ* hybridization analysis in tissue sections to visualize the changes in mRNA expression, and for solution hybridization assay to quantitate changes in mRNA levels. The results showed that within 6 h of hCG injection, there was a steady decline in the expression levels of LH receptor mRNA that reached almost non-detectable levels by 24 h (Hoffman et al., 1991; Lu et al., 1993; Peegel et al., 1994). This was followed by a gradual recovery to control levels by 72 h, as shown in the solution hybridization analysis data presented in Fig. 1. The loss of mRNA was rather unexpected, since the decrease of cell surface receptor expression was thought to occur through rapid internalization of the ligand-bound receptor. In the control samples an increase in LHR mRNA expression was seen during the time-course of the experiment, since our previous studies have shown that in pseudopregnant rat ovary the expression of LHR mRNA increased up to day 8 (Hoffman et al., 1991). The *in situ* hybridization pattern confirmed the Northern blot data and further suggested that the same corpora lutea that were depleted of the receptor mRNA reacquired the receptor

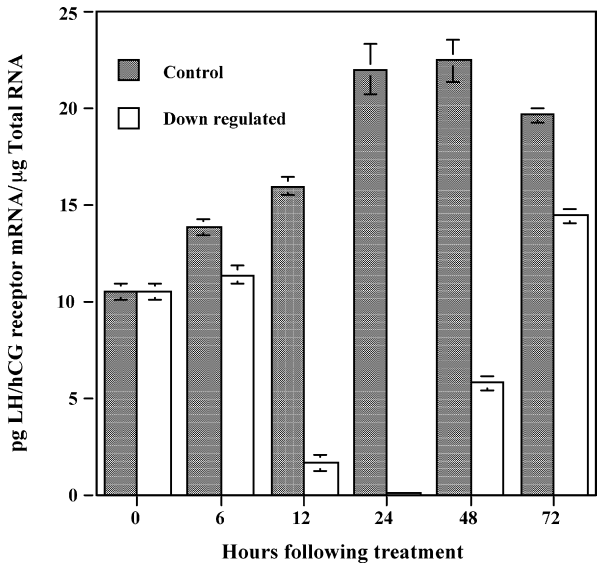

Fig. 1. LH/hCG-R mRNA levels during down-regulation time course. RNA was extracted from day 5 pseudopregnant control and down-regulated ovaries at 0, 6, 12, 24, 48, and 72 h after hCG injection. LH/hCG receptor levels were measured by solution hybridization assay. Total RNA (1–40 μg) was incubated with 1×10^4 cpm ^{35}S-labeled LH/hCG receptor antisense RNA. Single stranded RNA was digested by treatment with RNase, and the double stranded RNA was recovered by trichloroacetic acid precipitation. The concentration of LH/hCG receptor mRNA was extrapolated from a standard curve, generated using increasing concentrations of the unlabeled sense strand (0–200 pg), which exhibited reproducible linearity (modified from Peegel et al. (1994), Table 1 with permission from Endocrinology).

(Peegel et al., 1994). The loss of LH receptor mRNA was specific, as other LH/hCG responsive mRNAs such as Cytochrome p450scc remained at a level significantly higher than the saline treated controls (Hoffman et al., 1991). The loss of LH receptor mRNA occurred through post-transcriptional mechanisms as the transcription rate, determined by nuclear run on assays, remained identical in hCG treated group compared to the control. In fact, the nuclei isolated from the hCG treated ovaries was found to incorporate ^{3}H uridine into total RNA at much higher levels than that seen in control ovaries (Lu et al., 1993). Thus, we concluded that the loss of steady state LH receptor mRNA seen in the hCG treated group was due to increased mRNA degradation rather than a decrease in the rate of its synthesis.

3. Accelerated LH receptor mRNA degradation as a regulatory mechanism

It is now well recognized that the expression of specific, highly regulated mRNAs is regulated, at least in part, at the level of mRNA degradation. There are several examples where the mRNA degradation rate controls the steady state levels of mRNA expression (Ross, 1995; Sachs, 1993). In almost all instances where the mRNA expression is regulated by controlling its degradation, the changes in the stability of the mRNA have been shown to result from the binding of specific proteins to specific sequences and/or structures of the mRNA. The specific regions that the mRNA binding proteins interact with