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A novel post-transcriptional mechanism of regulation of luteinizing hormone receptor expression by an RNA binding protein from the ovary☆

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Abstract

Luteinizing hormone/human chorionic gonadotropin (LH/hCG) receptor, a member of the rhodopsin/β_2 adrenergic receptor subfamily of G-protein coupled receptors, is expressed primarily in the gonads and essential for the regulation of reproductive function. In the ovary, the expression of the receptor is post-transcriptionally regulated under physiological conditions. Studies from our laboratory showed that the ligand-induced down-regulation of the receptor occurs by accelerated degradation of the mRNA rather than by decreased transcription. We have identified a cytoplasmic LHR mRNA binding protein (LRBP) as a *trans*-acting factor in regulating LHR mRNA levels. LRBP binds to the coding region of LHR mRNA and causes accelerated degradation of mRNA. The RNA binding activity of LRBP was found to be inversely correlated to LH/hCG receptor mRNA levels. LRBP was purified to homogeneity and its identity was established as mevalonate kinase by N-terminal microsequencing and MALDI analysis. Mevalonate kinase, an enzyme involved in de novo synthesis of cholesterol, belongs to the GHMP family of kinases having a potential RNA binding fold. The expression of MVK mRNA and MVK protein levels were induced in response to hCG treatment prior to the down-regulation of LH/hCG receptor mRNA expression. A model for the post-transcriptional regulation of LH/hCG receptor in the ovary by mevalonate kinase is proposed.

Keywords: LH receptor; Mevalonate kinase; Coding region binding protein; Post-transcriptional regulation

1. Introduction

The receptor for the luteinizing hormone/human chorionic gonadotropin (LH/hCG) belongs to the glycoprotein subfamily of the rhodopsin β_2 adrenergic receptor-like family A of G-protein coupled receptors (Ascoli et al., 2002; McFarland et al., 1989). The rat receptor is composed of 675 amino acids consisting of a heavily glycosylated extracellular domain, a heptahelical transmembrane region and a short intracellular tail with sites for phosphorylation and palmitoylation (Ascoli et al., 2002). The LH/hCG receptors cloned from other species show significant sequence identity to the rat form of the receptor (Ascoli et al., 2002). The interaction of the ligand, LH or the placental hormone, hCG, results in the activation of adenylate cyclase to increase the intracellular levels of cyclic AMP which in turn stimulates steroidogenesis in the preovulatory follicle and the corpus luteum (Menon and Gunaga, 1974). It has also been shown that at high concentrations of hCG, the LH/hCG receptor activates the breakdown of PIP2 to DG and IP3 (Davis et al., 1984; Gundermann et al., 1992), although the physiological significance of this signaling pathway has not yet been fully elucidated.

Like other polypeptide hormone receptors, the LH/hCG receptor undergoes down regulation in response to ligand. This down-regulation can be seen both under physiological situations in response to the preovulatory LH surge as well as under pharmacological conditions when treated with a high dose of hCG (Hoffman et al., 1991; Lapolt et al., 1990; Peegel et al., 1994; Lu et al., 1993). The cells respond to these challenges by transiently down-regulating the cell surface expression of the receptors with a loss of cell response. Although down-regulation of the LH/hCG receptor occurs through multiple mechanisms including increased internalization of the ligand bound receptor, the most dramatic change occurred in the loss of steady state levels of LH/hCG receptor mRNA (Lu et al., 1993). Furthermore, the loss of steady state levels of the mRNA occurred post-transcriptionally by increasing the mRNA degradation rather than altering the transcription rate.

It is now becoming clear that the expression of highly regulated mRNAs such as c-fos, c-myc, LH/hCG receptor and the

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β_2 adrenergic receptor mRNAs are controlled at least in part at the level of mRNA degradation (Menon et al., 2004; Bernstein et al., 1992; Port et al., 1992; Sachs, 1993; Shyu et al., 1989). The stability of mRNA is regulated by sequence-specific interactions of specific mRNA binding proteins to either 5′ untranslated region as is the case with ferritin (Klausner et al., 1993), 3′ untranslated region in β_2 adrenergic receptor and granulocyte macrophage-colony stimulating factor. In other instances the binding site has been shown to be located in the open reading frame as is the case for c-fos and c-myc (Ross, 1995) and LH/hCG receptor mRNAs as described here. The ability to control mRNA level by regulating its degradation provides a fine control mechanism for the proper expression of genes involved in growth, differentiation and development. Here we describe our studies on the post-transcriptional regulation of LH/hCG receptor mRNA expression by a specific mRNA binding protein.

2. Effect of hCG on the steady state levels of LH/hCG receptor mRNA

The human and rat luteinizing hormone receptor genes are nearly 80 kb in size and consists of 11 exons (Ascoli et al., 2002). The exon 11 codes for the entire C-terminal and transmembrane region and a part of the extracellular domain whereas exons 1–10 code for the remaining N-terminal end (47 amino acids) of the extracellular domain of the rat LH receptor (Tsai-Morris et al., 1991). The 5′-flanking region of the rat LHR gene shows multiple transcriptional start sites. Transcription of rat LH/hCG receptor gene therefore results in multiple transcripts. In rat ovary, four LH/hCG receptor mRNAs of 6.7, 4.4, 2.6 and 1.8 kb in length have been identified (McFarland et al., 1989). All transcripts except the 1.8 kb contain the 2.1 kb open reading frame with varying length of 3′ untranslated region. Our studies have shown that the 6.7 kb transcript is the most abundant form of LH receptor mRNA in the ovary and is an extension of the 4.4 kb transcript in the 3′ untranslated region (Lu and Menon, 1994). The nucleotide identity of the 1.8 kb transcript remains unclear. It is postulated to encode an LH/hCG receptor lacking the transmembrane and C-terminal tail (Hu et al., 1994; Koo et al., 1991).

The expression levels of LH/hCG receptor on the cell surface varies during different physiological states of the ovary. In rat ovarian granulosa and luteal cells, the expression of LH/hCG receptor is greatly decreased by the endogenous preovulatory LH surge or by the administration of a pharmacological dose of human chorionic gonadotropin (hCG) (Kash and Menon, 1998; Lapolt et al., 1990; Hoffman et al., 1991; Peegel et al., 1994; Lu et al., 1993). We have shown that this decrease in cell surface expression of LH/hCG receptor seen in the ovary after hCG administration is paralleled by a specific transient disappearance of all four LH/hCG mRNA transcripts (Fig. 1) (Hoffman et al., 1991; Kash and Menon, 1998). During ligand-induced LH/hCG receptor down-regulation, all four rat LH/hCG receptor mRNAs were undetectable on a Northern blot at 12 h after hCG administration and began to reappear between 24 and 48 h post hCG injection (Kash and Menon, 1998). This dramatic

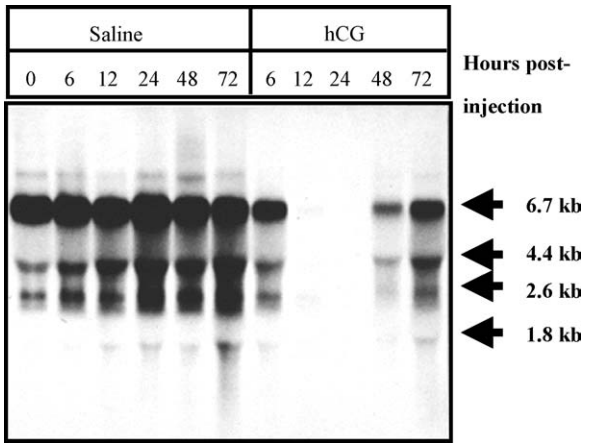

Fig. 1. Northern blot analysis of steady-state levels of LH/hCG receptor mRNA during hCG-induced LH/hCG receptor down-regulation. Total RNA was isolated from saline-injected or hCG-injected pseudopregnant rat ovaries. Sixty micrograms of RNA was loaded onto each lane. Blots were probed using a radiolabeled cDNA corresponding to the LH/hCG receptor carboxy terminus and a portion of the 3′ untranslated region (nucleotides 1936–2682) (modified from Kash and Menon [21], Fig. 1A; with permission from J. Biol. Chem.).

decline in LH/hCG receptor mRNA level upon hCG administration was found to be LH/hCG receptor specific as no decrease in mRNA levels of other genes were detectable (Hoffman et al., 1991). Since the transient disappearance of LH/hCG receptor mRNA levels in the ovary could be due to either a decrease in the rate of receptor gene transcription and/or an increase in the receptor mRNA degradation, further studies were carried out to determine transcription rate by nuclear run off assay and mRNA decay rate using solution hybridization analysis. Our results showed that the transient disappearance of LHR mRNAs did not result from any decreased transcription but occurred post-transcriptionally by a rapid degradation of LH/hCG receptor mRNA (Lu et al., 1993). We found a three-fold decrease in LH/hCG receptor mRNA half-life during the hCG-induced LH/hCG receptor down-regulated state in the rat ovary (Lu et al., 1993).

3. LH/hCG receptor mRNA binding protein

mRNA stability is governed by the interaction of various cytoplasmic/nuclear proteins (*trans*-acting factors) with the *cis*-acting regulatory regions in the mRNA (Ross, 1995). The formation and disruption of these ribonucleoprotein (RNP) complexes in response to various cellular stimuli mainly controls the stability of mRNA. A number of *trans*-acting factors have been identified as regulators of mRNA stability (Ross, 1995). In the ovarian system, we have examined the presence of potential *trans*-acting factors for the rat LH receptor mRNA that are responsible for the decrease in LH/hCG receptor mRNA stability during hormone-induced receptor down-regulation. We have identified an ovarian cytosolic protein named LH/hCG receptor mRNA binding protein (LRBP) in the 100,000 × *g* supernatant (S100) fraction prepared from the hCG-induced LH/hCG receptor down-regulated rat ovarian homogenate by an RNA elec-