Lutropin-Choriogonadotropin Receptor: An Unusual Member of the G Protein–Coupled Receptor Family

K. C. McFarland, Rolf Sprengel, Heidi S. Phillips, Martin Köhler, Nora Rosemblit, Karoly Nikolics, Deborah L. Segaloff,* Peter H. Seeburg

A complementary DNA (cDNA) for the rat luteal lutropin-choriogonadotropin receptor (LH-CG-R) was isolated with the use of a DNA probe generated in a polymerase chain reaction with oligonucleotide primers based on peptide sequences of purified receptor protein. As would be predicted from the cDNA sequence, the LH-CG-R consists of a 26-residue signal peptide, a 341-residue extracellular domain displaying an internal repeat structure characteristic of members of the leucine-rich glycoprotein (LRG) family, and a 333-residue region containing seven transmembrane segments. This membrane-spanning region displays sequence similarity with all members of the G protein–coupled receptor family. Hence, the LH-CG-R gene may have evolved by recombination of LRG and G protein–coupled receptor genes. Cells engineered to express LH-CG-R cDNA bind human choriogonadotropin with high affinity and show an increase in cyclic adenosine monophosphate when exposed to hormone. As revealed by RNA blot analysis and in situ hybridization, the 4.4-kilobase cognate messenger RNA is prominently localized in the rat ovary.

The lutropin-choriogonadotropin receptor (LH-CG-R), which is present on testicular Leydig cells and on ovarian theca, granulosa, luteal, and interstitial cells, plays a pivotal role in reproductive physiology. In the male and the nonpregnant female the LH-CG-R is exposed only to lutropin (LH), produced and secreted by the anterior pituitary. In the pregnant female, however, the ovarian LH-CG-R is also exposed to human choriogonadotropin (hCG), made by the placenta.

LH and hCG are members of a family of glycoprotein hormones which also includes thyroid-stimulating hormone (TSH) and follicle-stimulating hormone (FSH). All four are 28- to 38-kD heterodimeric glycoproteins composed of a common α subunit combined with distinct β subunits that confer receptor binding specificity (*1*). The carbohydrate moieties of these hormones appear to play an important role in signal transduction (*2*). The β subunits of LH and hCG are closely related in sequence, and these two hormones bind to the same receptor and elicit identical biological responses (*1*). The immediate response of target cells to the binding of LH and hCG is an increase in adenylyl cyclase activity mediated by intracellular, membrane-associated G proteins. The resulting increase in adenosine 3′,5′-monophosphate (cyclic AMP) ultimately leads to an increase in steroid synthesis and secretion (*3*). A family of G protein–coupled receptors has been identified whose members are characterized by the common structural feature of seven transmembrane domains known to be involved in signal transduction and in binding small ligands (*4*). In contrast, the LH-CG-R binds large, complex glycoproteins apparently via a large extracellular domain (*5*).

Progress toward the elucidation of the structure of the LH-CG-R has been hampered by the low abundance of this receptor and its susceptibility to proteolysis (*6*). Purification of the LH-CG-R from ovaries of pseudopregnant rats indicates that the purified receptor is a single glycoprotein with a molecular mass of 93 kD (*7*). Although this conclusion is in agreement with our earlier results (*8*) and those of several others (*6*), some data have suggested that the LH-CG-R may be composed of multiple subunits (*6*).

For a clear understanding of the structure and function of the LH-CG-R, cloning of the receptor cDNA is necessary. We now describe the molecular cloning and expression of a full-length cDNA for the rat luteal LH-CG-R, which encodes a single polypeptide that binds hormone and stimulates adenylyl cyclase. Localization of the receptor mRNA in the ovary corresponds to that of hormone binding. The deduced amino acid sequence suggests that the LH-CG-R is related to other G protein–coupled receptors in that it contains seven transmembrane regions. However, unlike other such receptors, the LH-CG-R contains a large extracellular domain that is presumably involved in ligand binding.

Protein sequencing, molecular cloning, and structure of the LH-CG-R. LH-CG-R purified by lectin and hCG affinity chromatography (*7*) was further purified by SDS–polyacrylamide gel electrophoresis (PAGE) and used to obtain the NH_2-terminal amino acid sequence. Several internal sequences were also obtained

K. C. McFarland, H. S. Phillips, and K. Nikolics are in the Department of Developmental Biology, Genentech, Inc., South San Francisco, CA 94080. R. Sprengel, M. Köhler, and P. H. Seeburg are at the Zentrum for Molekulare Biologie Heidelberg, University of Heidelberg, F.R.G. N. Rosemblit and D. L. Segaloff are at The Population Council, New York, NY 10021. K. C. McFarland and R. Sprengel should be considered authors with equal contribution.

*To whom correspondence should be addressed at The Population Council, 1230 York Avenue, New York, NY 10021.

from peptide fragments generated by chemical and enzymatic cleavages (Table 1 and Fig. 1). Oligonucleotides based on the NH_2-terminal peptide and one internal peptide were used successfully as primers in a polymerase chain reaction (PCR) (*9*) with rat luteal cDNA as a template. A distinct and prominent DNA product of 622 base pairs was generated; it contained an open reading frame encoding additional peptide sequences that had been determined previously from receptor fragments. This PCR product was used as a probe for screening a rat luteal cDNA library (*10*).

The nucleotide and predicted amino acid sequence of the rat LH-CG-R cDNA with 43 nucleotides of 5′ flanking and 759 nucleotides of 3′ flanking sequence are shown in Fig. 1. The translation initiation codon at position 1 defines the start of a 2100-nucleotide long open reading frame that encodes all independently determined peptide sequences. A putative signal peptide (*11*) of 26 residues precedes the peptide determined from the uncleaved LH-CG-R polypeptide. Hence, we predict that the mature LH-CG-R begins with arginine and is composed of 674 amino acid residues (75 kD).

The NH_2-terminal half of the polypeptide sequence (residues 1 to 341) is hydrophilic and presumably constitutes the extracellular domain (Fig. 2). Consonant with the glycoprotein nature of the LH-CG-R, there are six potential sites for N-linked glycosylation within this domain. Initial evidence suggests that most of these sites are indeed glycosylated (*12*) and this may account for the difference in molecular size between the natural LH-CG-R (93 kD) and the predicted mature unglycosylated polypeptide (75 kD).

The COOH-terminal half of the polypeptide (residues 342 to 674) contains seven hydrophobic segments of membrane-spanning

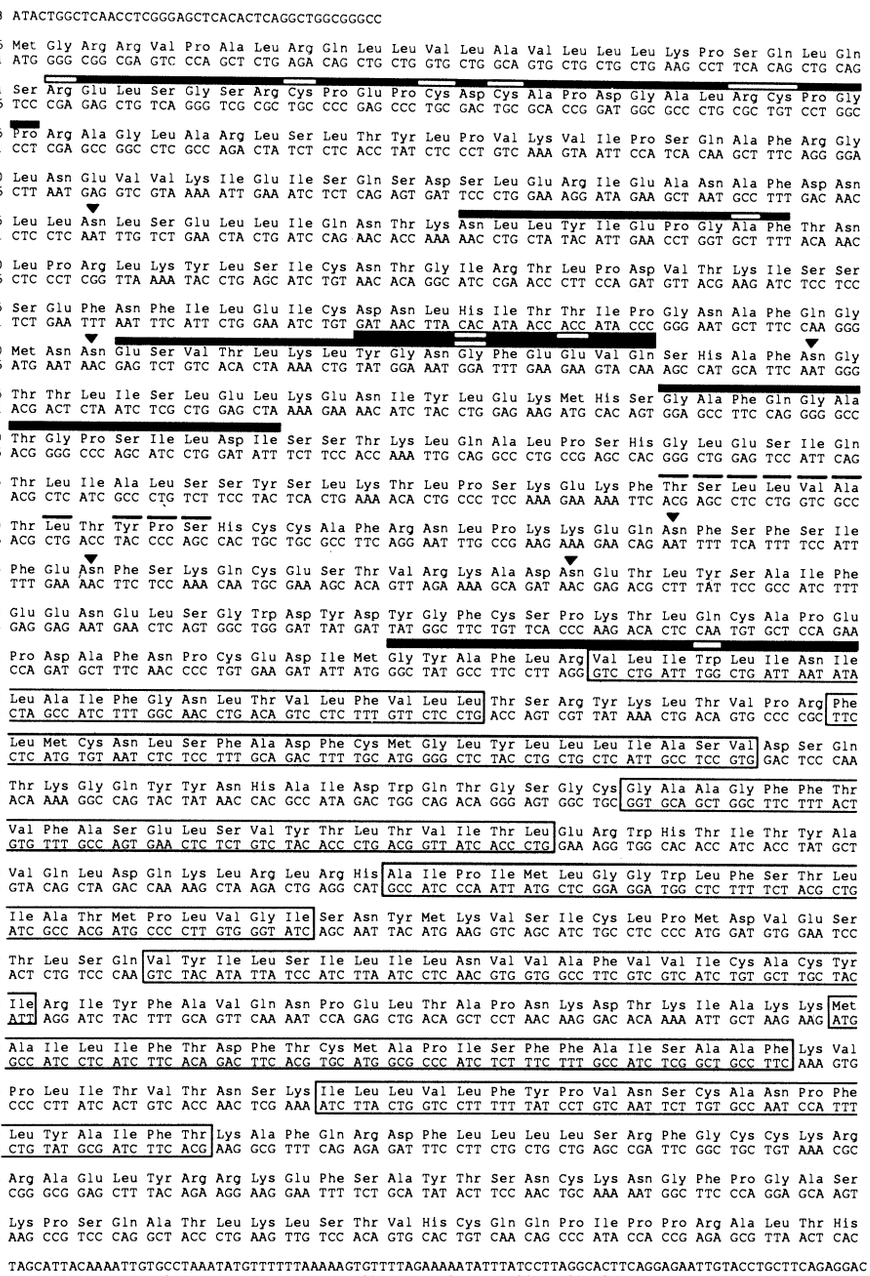

Fig. 1. The cDNA and predicted amino acid sequence of rat ovarian LH-CG-R. Chemically determined peptide sequences (Table 1) are indicated by bars above corresponding sequences, with residues differing from those predicted indicated by white bars. Amino acid numbering begins at the NH_2-terminal sequence for the mature intact receptor, with negative numbers for the encoded signal sequence. Putative sites for N-linked glycosylation are marked by inverted triangles, and the proposed membrane-spanning hydrophobic sequences are enclosed in boxes. Thin lines above residues show location of similarity to soybean lectin (*20*). The LH-CG receptor was purified from the ovaries of pseudopregnant rats as described (*7*), except that the wheat germ agglutinin chromatography was performed before the hCG-affinity chromatography. Lectin and affinity purified receptor was concentrated by Centricon-30 (Amicon) and resolved by PAGE under nonreducing conditions. The resolved 93-kD receptor band was electroblotted onto PVDF (Millipore) membranes (*26*), and the mature NH_2-terminal sequence was determined by gas-phase microsequencing (*27*). The 93-kD receptor was also electroeluted and was cleaved by cyanogen bromide or lysyl–C endopeptidase. The fragments were subjected to high-performance liquid chromatography (HPLC) or tricine gels (*28*), electroblotted, and analyzed by gas-phase microsequencing. The oligonucleotides constructed from these peptide sequences were as follows: ks (from peptide lhrk), AAGGAGCTG(AG/TC)TGGC-(AG/TC)C(C/A)GGAAGCCTGAGCCCAA-TGACTTCGCCCCTGATGGTGCCCT; rsrc (from lhrr, reverse complement), TGCACCTCC-TCGAAGCAGTTGCCATACAGCTTCAG-GGTCACA(CT/GA)CTC and fsrc (from lhrf, reverse complement), ACG(GG/AT)GTCCAGGA-TG(CT/GA)AGGGCC(T/A)GTGGCACCC-TGGAAGGC(T/C)CC. Oligonucleotide mixtures of ks with rsrc and of ks with fsrc (500 ng each) were used for priming polymerase chain reactions (PCR) with cDNA (25 ng) synthesized from pseudopregnant rat ovarian poly(A)$^+$ RNA (*29*). Upon sequence analysis (*30*), a 622-bp fragment amplified by ks with fsrc was found to contain part of the LH-CG-R coding sequence, including the sequences for additional peptides lhrr, lhr26, and lhr28. This fragment was used to probe a cDNA library (10^6 recombinant phage) constructed in λgt10 (*31*) from pseudopregnant rat ovarian RNA. Of 20 hybridizing phage, 13 were further analyzed by DNA sequencing and used to determine the nucleotide sequence of the LH-CG-R cDNA.

length and displays sequence homology to all members of the G protein–coupled receptor family (see below). If the transmembrane topology is identical to that suggested for rhodopsin (*13*), the COOH-terminal 68 residues of the LH-CG-R would be intracellular. This COOH-terminal domain contains potential phosphorylation sites (serine, threonine, and tyrosine residues) where cellular control of receptor activity may occur (*14*). This domain also contains two clusters of basic amino acids (at positions 623 to 625 and 630 to 632) that represent potential sites for proteolytic cleavage (*15*). However, it is not known whether the mature receptor may be post-translationally cleaved to terminate at one of these positions.

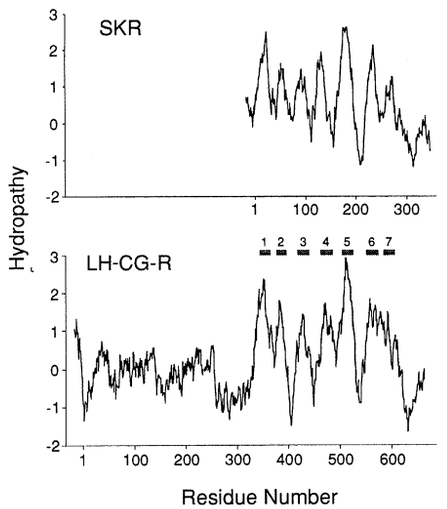

Fig. 2. Hydropathy plot of LH-CG-R. A Kyte and Doolittle hydropathy profile of the substance K receptor (SKR, upper panel) and LH-CG-R (lower panel), with a window of 20 residues (*32*). The alignment of the SKR and LH-CG-R in the region of the putative transmembrane domain shows the pattern of hydropathy found in the G protein–coupled receptor family. The seven putative transmembrane regions for the LH-CG-R are marked by the numbered solid bars.

The homology of the COOH-terminal half of the LH-CG-R to other members of the G protein–coupled receptor family is superficially revealed by a hydropathy plot (Fig. 2). This homology is revealed in more detal in the seven putative transmembrane regions by an alignment with several members of this family (Fig. 3) (*16*). This domain of the LH-CG-R shows a low but significant sequence similarity to other members. Similarity in the regions shown in Fig. 3 is higher to the β2 adrenergic receptor (26 percent identity) and lower to other receptors in this famly (18 to 24 percent) (*17*). However, comparison of the full-length sequences shows greater overall identity with rhodopsin and substance K receptor (SKR) (22 percent) than with β2 adrenergic receptor or the other neurotransmitter receptors (18 to 21 percent) (Fig. 3).

A number of short sequences are highly conserved in all members and occur within the putative transmembrane helices and intracellular loop regions. However, no functional role can be assigned to these conserved structures. One of the conserved sites spans six or seven residues located on the COOH-terminal end of the third cytoplasmic loop. This loop varies in its length between different receptors, being shortest in LH-CG-R. On the basis of analysis of mutant and chimeric receptors, the COOH-terminal region of this loop has been implicated in G protein–coupling (*18*). However, this site is not more conserved in sequence between LH-CG-R and other receptors that couple to G_s than between LH-CG-R and receptors known to interact with other G proteins.

The extracellular domain displays several significant sequence features. Sequence comparison reveals that the protein most similar to the extracellular domain of the LH-CG-R is PG40, a proteogly-

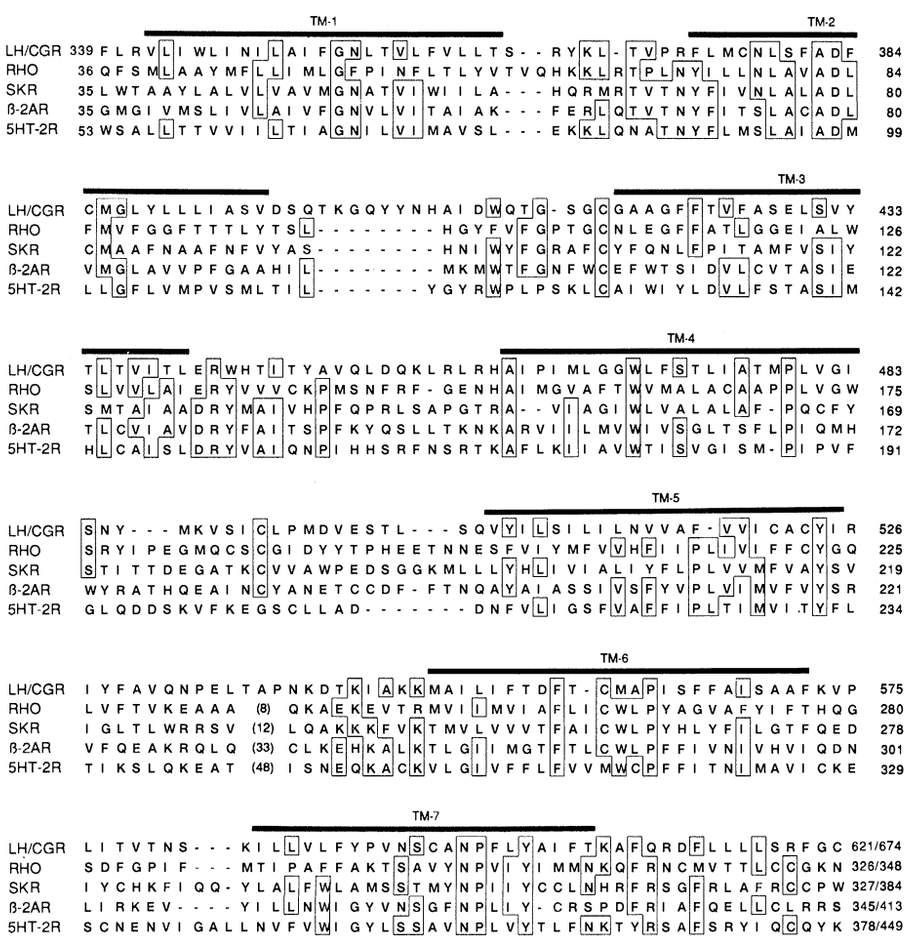

Fig. 3. Alignment of the transmembrane regions of LH-CG-R. The transmembrane regions of selected G protein–coupled receptors were aligned by fastp (*33*) and homglobal (*17*) computer programs, with final adjustment made by hand to maximize positional identity with minimal insertions. Numbers denote residue number; numbers in parentheses show the number of residues deleted in the five-to-six loop region. Boxed regions show matches of three or more residues at each position. Numbered bars indicated positions of putative transmembrane (TM) regions. RHO, bovine rhodopsin; SKR, substance K receptor; β-2AR, β2 adrenergic receptor; and 5HT-2R, 5HT-2 (serotonin) receptor (*16*). The similarity between such distant relatives of a protein family are sometimes best described by their homglobal-derived "z-score" (*17*). Overall scores for these receptors: Rhodopsin, 15.05; SKR, 12.95; β-2AR, 7.84; and 5HT-2R, 3.53.

A

	(1-11)	R E L S G S R C P E P
I	(12-38)	C D C A P D G - - A L R C P G P R A G L A R L S L T Y L P -
II	(39-63)	V K V I P S Q - - A F R G L N E V V - K I E I S Q S D S - -
III	(64-88)	L E R I E A N - - A F D N L L N L S - E L L I Q N T K N - -
IV	(89-112)	L L Y I E P G - - A F T N L P R L K - Y L S I C N T G - - -
V	(113-138)	I R T L P D V - - T K I S S S E F N F I L E I C D N L H - -
VI	(139-164)	I T T I P G N - - A F Q G M N N E S V T L K L Y G N G - - -
VII	(165-187)	F E E V Q S H - - A F N G T T L I S - - L E L K E N I Y - -
VIII	(188-211)	L E K M H S G - - A F Q G A T G P S - I L D I S S T K - - -
IX	(212-233)	L Q A L P S H - - - - - G L E S I Q T L I A L S S Y S - - -
X	(234-251)	L K T L P S K - E K F T S L L V A T L - - - - - - - - - - -
XI	(252-273)	- - T Y P S H C C A F R N L P K K E Q N F S F S - - - - - -
XII	(274-290)	- - - - - - - - - - I F E N F S K Q C E S T V R K A D - - - -
XIII	(291-318)	N E T L Y S A - - I F E E N E L S G W D Y D Y G F C S P K T
XIV	(319-341)	L Q C A P E P - D A F N P C E D I M G Y A F L R - - - - - -

B

LRG	L x x L P x x - - L L x x L x x L x - x L D L S x N x
Toll	L x x L P x x - - L F x H x x N L x - x L x L x x N x
GPIB	L T T L P x G - - L L x x L P x L x - x L x L S x N x
ACY	a x x a P x x - - - a x x L x x L x - x L x L x x N x

Fig. 4. Structure of the repetitive motif in the extracellular domain of the LH-CG-R. (**A**) Alignment of the 14 imperfect repeat structures. Identical or conserved residues among the segments I–XIV have been boxed. Dashes indicate the placement of gaps to optimize the periodicity. (**B**) Consensus sequences for the leucine-rich repetitive motifs observed in the leucine-rich α_2-glycoprotein of human serum (LRG), the α chain of human platelet glycoprotein Ib (GP1B), the Toll developmental gene product of *Drosophila* (Toll), and the yeast adenylyl cyclase (ACY) (*19*). The letter a indicates one of three aliphatic amino acids valine, leucine, or isoleucine.

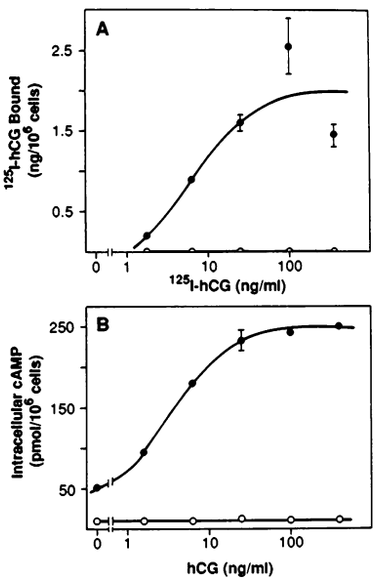

Fig. 5. Functional expression of the LH-CG-R cDNA. Specific ^{125}I-labeled hCG binding (A) and hCG-stimulated cyclic AMP accumulation (B) in 293 cells transiently transfected with (•) or without (○) expression vector pCLHR. The expression vector pCLHR was constructed by introducing the entire coding region of the cloned cDNA and additional flanking regions contained on an Eco RI fragment (nucleotides −43 to 2559; see Fig. 1) into the pCIS vector (*21*). Exponentially growing 293 cells (in 60-mm dishes) were transfected (*22*) with this expression vector; 42 hours later intact cells were assayed for ^{125}I-labeled hCG binding (A) or hCG-stimulated cyclic AMP production (B). (**A**) Each dish was washed four times with 3 ml of warm Waymouth MB752/1 medium lacking sodium bicarbonate and containing 20 m*M* Hepes and bovine serum albumin (BSA) at 1 mg/ml and then placed in 2 ml of the same medium. After 2 hours at 4°C, portions of highly purified hCG (CR-123, 12,780 IU/mg) that had been iodinated as described (*8*) were added alone or together with 50 IU of crude hCG (for the determination of nonspecific binding). After 24 hours at 4°C, the binding medium and cells were transferred to plastic tubes on ice. The cells were centrifuged, washed once with 2 ml of cold 150 m*M* NaCl, 20 m*M* Hepes containing BSA at 1 mg/ml, and centrifuged again. The radioactivity in the pellet was counted in a gamma counter. Results shown are corrected for nonspecific binding and represent the mean ± the range of duplicate determinations. (**B**) Each dish was washed four times with 3 ml of warm Waymouth MB752/1 medium containing BSA at 1 mg/ml and placed in 2 ml of the same containing 0.5 m*M* 3-isobutyl-1-methylxanthine. After a 15-minute incubation period at 37°C, highly purified hCG was added and the incubation was continued for 30 minutes at 37°C. After the assay medium was removed, the cells were collected into 1.5 ml of cold 1*N* perchloric acid containing theophylline at 1 mg/ml. Cells were lysed by rapid freezing and thawing and then centrifuged. The supernatants were neutralized (*34*) and then assayed for cyclic AMP (*35*). Each data point represents the mean ± the range of duplicate determinations.

can abundant in extracellular matrices of connective tissues that binds collagen (*19*). The most striking feature of the extracellular domain of LH-CG-R is a 14-fold imperfectly repeated sequence of approximately 25 residues (Fig. 4A). Of these, the COOH-terminal six repeats are the least conserved in length and sequence. Similar motifs, which have been termed leucine-rich repeats, have been recognized in various proteins comprising the family of leucine-rich glycoproteins (LRG) (Fig. 4B) (*19*). The LRG family includes such widely divergent polypeptides as yeast adenylyl cyclase, the α_2-serum glycoprotein, the *Drosophila* Toll developmental gene product, and platelet glycoprotein 1b. The latter is interesting because it is a glycosylated membrane protein that binds two glycosylated polypeptides, von Willebrand factor and thrombin. It is now recognized that PG40 is also a member of the LRG family (*19*).

Members of the LRG family have been postulated to interact with both hydrophobic and hydrophilic surfaces, possibly mediated by amphipathic helices formed by the repeat structures. Since the large extracellular domain of the LH-CG-R is apparently responsible for binding LH and hCG (*5, 8*), this domain may be responsible both for binding of hormone and for interacting with the transmembrane domains to mediate signal transduction.

Another feature within the extracellular domain constitutes a site defined by ten residues that are identical to a sequence present in soybean lectin (*20*) (Fig. 1). Although deglycosylated LH and hCG bind to the receptor with high affinity, this interaction results in little or no stimulation of adenylyl cyclase (*2*). Thus, this site may be involved in the recognition of the glycosylated hormone and the functional coupling of the receptor to G_s, maximally achieved only with the glycosylated forms of LH and hCG.

Functional expression of the LH-CG-R. To confirm that the cloned cDNA indeed encodes the LH-CG-R, we constructed the expression vector pCLHR in which the putative receptor coding sequence is under the transcriptional control of the cytomegalovirus promoter (*21*). Cells from human embryonic kidney, termed 293 cells, were transiently transfected (*22*) with pCLHR and tested for

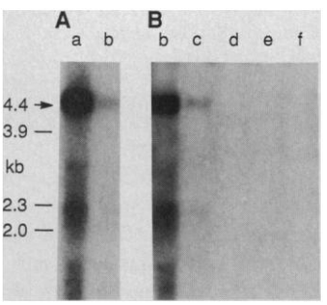

Fig. 6. RNA analysis of the hybridization of LH-CG-R cDNA in different tissues. Each lane contained 10 μg of total RNA. Numbers on the left indicate DNA size markers in kilobase. Samples shown are from the ovaries of pseudopregnant rats (lane a), and adult rat ovaries (lane b), testes (lane c), lung (lane d), liver (lane e), and kidney (lane f). (**A**) and (**B**), respectively, are 6-hour and overnight exposures of the same blot. Lane a is shown only as a 6-hour exposure, since the overnight exposure of this lane was overexposed. Total RNA was prepared (*36*) from the ovaries of immature rats rendered pseudopregnant (*7*) or from tissues of 60-day-old rats. The RNA was resolved on 1 percent agarose gels containing formaldehyde and blotted onto a nylon membrane (ICN). Manufacturer's procedures were followed: a preliminary hybridization preceded hybridization overnight at 42°C with a nick-translated ^{32}P-labeled pGEM-3Z vector (Promega) containing the 622-bp PCR-generated LH-CG-R DNA fragment. The blot was washed four times in 2×SSC (saline sodium citrate) and 0.1 percent SDS at room temperature (5 minutes per wash) and then two times in 0.1×SSC and 0.1 percent SDS at 65°C (30 minutes per washing). The resulting blot was exposed for 6 hours (A) or overnight (B) to x-ray film at −70°C with intensifying screens.

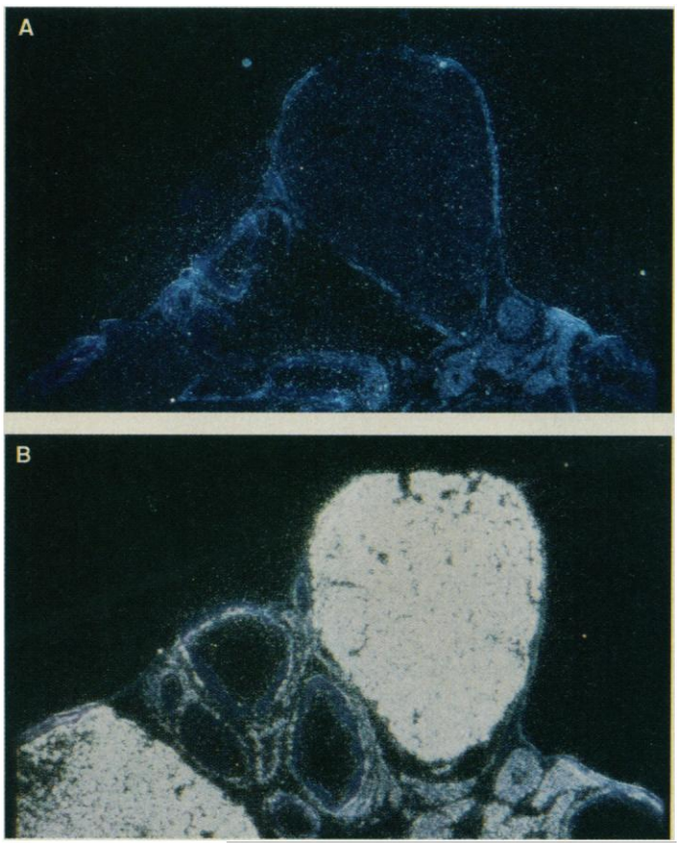

Fig. 7. Dark-field microscopy of in situ hybridization in rat ovary. (**A**) A 10-μm section from an ovary of a 9-day pregnant rat was hybridized with a ^{35}S-labeled probe that was sense to the 622-bp PCR-generated LH-CG-R DNA fragment. (**B**) The adjacent section was hybridized with a labeled antisense strand. The tissue was fixed and hybridized in situ by the method of Wilcox *et al.* (*37*) with the following modifications. Prior to hybridization, the sections were treated with 4 percent paraformaldehyde for 10 minutes and then with proteinase K at 5 to 10 μg/ml for 5 to 10 minutes. A preliminary hybridization was performed for 1 hour at 42°C in 100 μl of hybridization buffer containing 50 percent formamide, 0.1*M* NaCl, 20 m*M* tris, *p*H 8.0, 5 m*M* EDTA, 1× Denhardt's solution, 10 percent dextran sulfate, and 10 m*M* dithiothreitol. Hybridization was initiated by the addition of a ^{35}S-labeled probe (6×10^5 cpm) in 20 μl of buffer and proceeded overnight at 55°C. The ^{35}S-labeled sense and antisense probes were obtained from the 622-bp PCR-generated LHR-CG-R DNA fragment cloned into pGEM-3Z vector (Promega). The specific activity of the probes was approximately 100 Ci/mmol. Exposure times were 1 to 3 weeks (micrographs displayed are 2-week exposures).

Fig. 8. A postulated model for the LH-CG-R in the plasma membrane. Above the membrane bilayer is extracellular space; below is cytoplasmic.

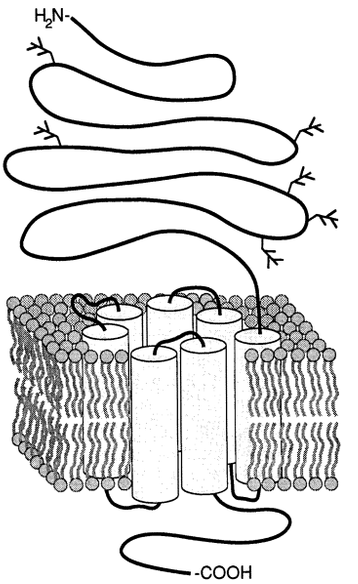

Table 1. Sequences determined from purified LH-CG-R peptides. Sequence lhrk was determined from a CNBr fragment as well as from the intact receptor. Residues shown in parentheses are ambiguously identified residues. Undetermined residues are marked by an X. The sequences used for primers in the successful PCR are underlined.

Peptide	Sequence derived from cleavage
	From CNBr
lhrk	KELSGSRKEPEPNDFAPDGALXXPGP
lhrr	MXXESVTLKLYGNCFEEVQ
lhrf	MX(S)GAFQGATGPSILD(P)V (E) (I)
lhrc	M(D)YA(G)LXVLI(G)LINILDXF (G) (F) (F) (A) (N)
	From lysyl C-endopeptidase
lhr26	KXYGNXFEXVQ
lhr28	KNLLYIEPGSF

their ability to bind hCG and respond to hCG with an increase in cyclic AMP.

Intact transfected cells exposed to increasing concentrations of ^{125}I-labeled hCG specifically bound hormone in a concentration-dependent and saturable manner (Fig. 5A). No specific binding was observed to untransfected cells at any concentration of ^{125}I-labeled hCG tested. A parallel group of cells was incubated with varying concentrations of hCG in the presence of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine. In contrast to untransfected cells, which showed no elevation of cyclic AMP in response to hCG, transfected cells displayed a concentration-dependent and saturable increase in intracellular cyclic AMP when exposed to hCG (Fig. 5B).

The concentrations of hCG required to elicit half-maximal binding (about 8 ng/ml, or 208 p*M*) and half-maximal stimulation of cyclic AMP accumulation (about 2.5 ng/ml, or 65 p*M*) in cells transfected with pCLHR are comparable to those reported in LH-CG-R–bearing gonadal cells (*23*). The above results demonstrate that the cloned cDNA encodes for a functional LH-CG-R protein.

Tissue and cell specific expression of LH-CG-R mRNA. RNA blots prepared from the RNA's of different rat tissues demonstrated tissue specificity of LH-CG-R mRNA expression (Fig. 6). A distinct band corresponding to a 4.4-kb mRNA was observed in RNA prepared from the ovaries of pseudopregnant rats as well as from ovaries and testes of adult rats. Smaller hybridizing species at 1.8- and 1.2-kb were also observed during longer exposure times. The relative abundance of the 4.4-kb mRNA species was greater in the ovaries of pseudopregnant rats than in the ovaries of nonpregnant adult female rats or the testes of adult rats. This finding is consistent with relative levels of ^{125}I-labeled hCG binding in these tissues (*6*). No LH-CG mRNA was observed in RNA prepared from rat lung, liver, or kidney.

To analyze cell specific expression of LH-CG-R mRNA, we performed in situ hybridization of the LH-CG-R cDNA to tissue slices prepared from ovaries of 9-day pregnant rats. Prominent hybridization of the labeled antisense strand was observed to the corpora lutea and to the theca and interstitial cells (Fig. 7). No hybridization to the granulosa cells was seen, consistent with the immature state of the nonluteinized follicles. The observed distribution and relative intensities of hybridizing mRNA (such as intense staining of the corpora lutea and less intense staining of theca and interstitial cells) are consistent with the localization of ^{125}I-labeled hCG binding in the rat ovary as determined by autoradiography (*24*). These findings provide further evidence that the cloned cDNA

encodes the functional LH-CG-R expressed in specific subsets of ovarian cells.

In summary, we have presented functional and morphological evidence that the protein sequence encoded by the cloned cDNA represents the rat ovarian LH-CG-R (Fig. 8). This protein displays the structural features of both a leucine-rich proteoglycan (extracellular domain) and of a G protein–coupled receptor. The other members of the G protein–coupled receptor family bind small ligands (such as serotonin or acetylcholine). Whereas binding of such ligands is thought to occur at sites formed by the assembly of the seven transmembrane helices (*25*), LH and hCG are thought to bind to a site on the extracellular part of this receptor (*5, 8*). Thus, the large extracellular domain and the specific mechanism of hormone-mediated signal transduction set the LH-CG-R apart from other G protein–coupled receptors. This receptor may have originated by recombination of genes encoding a hormone-binding glycoprotein and a seven-transmembrane proto-receptor.

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