Published in final edited form as:

Mol Cell Endocrinol. 2007 January 2; 260-262: 126–136.

A functional transmembrane complex: The luteinizing hormone receptor with bound ligand and G protein

D. Puett^a, Y. Li^a, G. DeMars^a, K. Angelova^a, and F. Fanelli^b

^a Department of Biochemistry & Molecular Biology, University of Georgia, Athens, GA 30602, USA

^b Dulbecco Telethon Institute and Department of Chemistry, University of Modena and Reggio Emilia, via Campi 183, 41100 Modena, Italy

Abstract

The luteinizing hormone receptor (LHR) is one of eight members in a cluster of the rhodopsin family of the large G protein-coupled receptor (GPCR) superfamily that contains some 800–900 genes in the human genome. LHR, along with its paralogs, follicle stimulating hormone receptor (FSHR) and thyroid stimulating hormone receptor, form one of the three classes in this cluster; the two other classes contain the relaxin-binding GPCRs and orphan GPCRs. These GPCRs are characterized by a relatively large ectodomain (ECD) containing leucine-rich-repeats (LRRs); in the class of glycoprotein hormone receptors, the LRR region is capped by N-terminal and C-terminal cysteine-rich regions. Binding of human chorionic gonadotropin (hCG) or luteinizing hormone to the LHR-ECD triggers a conformational change of the transmembrane region of the receptor facilitating binding and activation of Gs, followed by effector enzyme activation and subsequent intracellular signaling. Viewing LHR as a transmembrane anchoring protein that sequentially binds hCG and Gs to give the hCG-LHR-Gs complex, numerous interactions and conformational changes must be considered. There is, unfortunately, a paucity of structural data on LHR, but crystal structures exist for hCG, the homologous FSH-FSHR-ECD (N-terminal fragment) complex, rhodopsin (in the inactive state), an active form of G α s (transducin), and the $\beta\gamma$ heterodimer. Using a combined experimental (site-directed mutagenesis followed by characterization in transfected cells) and computational (homology modeling and molecular dynamics simulations) approach, good working models are being developed for the protein-protein interaction faces and, in some cases, the ensuing conformational changes induced by complex formation. hCG binding to the LHR-ECD appears to involve several LRRs; LHR activation can be described in terms of disrupting a network of H-bonds in the cytosolic halves of helices 1–3, 6, and 7; and binding of LHR to Gs involves, in large part, intracellular loop 2 binding, presumably to G α s at its C-terminus. Major gaps exist in our understanding at the molecular level of the six-polypeptide chain complex, hCG-LHR-Gs, but considerable progress has been made in the past few years.

Keywords

Human chorionic gonadotropin; LH receptor; Gs protein; Gonadotropin-mediated signaling; G protein-coupled receptor

*Corresponding author: Department of Biochemistry & Molecular Biology, Life Sciences Building, University of Georgia, 120 Green Street, Athens, GA 30602-7229, USA. Tel.: 706-542-1676; fax: 706-542-0181; e-mail address: puett@bmb.uga.edu..

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

The luteinizing hormone (LH) receptor (LHR) binds two ligands, LH and chorionic gonadotropin (CG), with high affinity and high specificity (Hearn and Gomme, 2000). As a member of the G protein-coupled receptor (GPCR) superfamily, which constitutes roughly 1% of the human genome (Takeda et al., 2002), LHR belongs to the glycoprotein hormone receptor (GPHR) cluster within the rhodopsin family. This cluster also contains the follicle stimulating hormone (FSH) receptor (FSHR), the thyroid stimulating hormone (TSH) receptor (TSHR), and five other GPCRs (Fredriksson et al., 2003). These eight receptors constitute three classes, all of which contain a relatively large leucine-rich-repeat (LRR) ectodomain and some of which are capped by cysteine-rich N-terminal and C-terminal regions. LHR and FSHR are located on chromosome 2, while the other six GPHRs in the cluster are on six distinct chromosomes (Fredriksson et al., 2003).

The basic architecture of all GPCRs includes seven transmembrane helices, three extracellular and three intracellular loops, and, in most, an intracellular C-terminal tail. While there is considerable physiological and pharmacological interest in the GPCRs, the only structure available is that for rhodopsin (Palczewski et al., 2000). This structure has thus served as a template for modeling LHR (Fanelli et al., 2001, 2004; Fanelli and Puett, 2002; Angelova et al., 2002; Ascoli et al., 2002) and other GPCRs.

Recently, a crystal structure became available for a complex of single-chain FSH bound to a large N-terminal fragment (residues 1–268) of the FSHR ectodomain (ECD) (Fan and Hendrickson, 2005a). Interestingly, there are some differences in the structures of unbound FSH (Fox et al., 2001) and receptor-bound FSH (Fan and Hendrickson, 2005a), particularly in the α -subunit C-terminal region. With the recent advances in protein structure prediction, e.g. via threading and comparative modeling, coupled with the known crystal structure of hCG (Lapthorn et al., 1994; Wu et al., 1994; Tegoni et al., 1999), there is an opportunity to develop reasonable working models of the LHR-ECD based on homologous proteins of known structure, with and without bound hCG or hLH. These predicted structures can now be tested against the crystal structure of the FSH-FSHR 1–268 complex (Fan and Hendrickson, 2005a). Such a computational approach with hCG and LHR can advantageously use the available experimental data, derived mainly from site-directed mutagenesis of ligands and receptor, to guide the development of plausible models. This review covers the current status of this area and suggests major topics that are in need of further research.

2. Overview of the General Problem

A model of the hCG-LHR-Gs complex is shown in Fig. 1 for illustrative purposes only; indeed, some of the regions are based on structural data, others on molecular modeling, and some on little more than artistic freedom. Each of the following topics will be addressed in subsequent sections, and thus the following is intended as an overview. While the exact details need to be determined, a combination of experimental and computational data gives some degree of confidence in the nature of the ligand-ECD complex. Likewise, the overall features of the transmembrane portion are probably correct. The major areas requiring elucidation are the mode of activation of the transmembrane region by the hormone-ECD complex, the nature of ligand-induced reorientation of the transmembrane helices, and the mechanism by which the activated receptor binds to and activates Gs, i.e. GTP-GDP exchange of Gs α followed by dissociation from $\beta\gamma$.

3. The LHR-ECD: Structure and Hormone Binding

Based on the pseudo-LRR nature of much of the LHR-ECD and the structure of the LRR protein, ribonuclease inhibitor (Kobe and Disenhofer, 1993), as a template, several laboratories

proposed models for the LHR-ECD with 9–14 LRRs (Moyle et al., 1995; Kajava et al., 1995; Jiang et al., 1995; Bhowmick et al., 1996; Couture et al., 1996; Song et al., 2001a), the former of which now appears to be more accurate. Using the threading program PROSPECT (Kim et al., 2003) and the SCOP40 database (Chandonia et al., 2004), we comparatively modeled the LHR-ECD prior to the publication of the FSH-FSHR-ECD complex. The amino acid sequence was first analyzed by PSI-BLAST in order to identify conserved amino acid residues in different species, the sequence profile then being included in the threading. Significant z-scores were obtained with two proteins, glycoprotein Ib α (Huizinga et al., 2002) and the Nogo receptor (He et al., 2003). These two proteins contain, respectively, seven and eight LRRs like those of the LHR-ECD: LxxLxLxxNxxLxxLPxxaFxxLxx (see Fig. 2 for a typical structure) where L refers generally to Leu, Ile, or Val, N denotes Asn and occasionally Ser or Thr, P and F are Pro and Phe, respectively, “a” corresponds to an amino acid residue with a small side chain, and “x” represents any amino acid residue. Also like the LHR-ECD, these two proteins have N-terminal and C-terminal cysteine-rich regions that form two caps to shield the hydrophobic core of the LRR region.

The model shown in Fig. 3A for the LHR-ECD was generated using the MODELLER program (Fiser and Sali, 2003). The LRRs form a more open structure than that originally proposed (Bhowmick et al., 1996) and contain proposed structures for the N- and C-terminal cysteine-rich regions, characterized, respectively, by the presence of two antiparallel β strands and α helices/parallel β strand that is aligned to the last β strand in the LRR.

Following the same protocol, residues 1–241 of the FSHR-ECD were modeled with the results given in Fig. 4A. The crystal structure of FSHR-ECD 1–241, devoid of the bound FSH (Fan and Hendrickson, 2005a), is shown in Fig. 4B, and a superposition of the modeled and experimentally determined structures is presented in Fig. 4C. The RMSD between the model and the crystal structure of the FSHR-ECD 18–247 is 2.7 Å for the C α atoms and 3.5 Å for all atoms. Clearly, the computational approach as used yielded a rather good prediction of the actual structure. The crystal structure of the FSHR-ECD was then used as a template to model the LHR-ECD (residues 1–241), and the results are shown in Fig. 3B. A comparison of the predicted structure of the LHR-ECD (residues 1–241), based on using the Nogo receptor and glycoprotein Ib α as templates (Fig. 3A), with that obtained using the paralogon FSHR-ECD structure as a template (Fig. 3B), demonstrates excellent agreement. The major areas where discrepancies arise are at the beginning of LLR5, in which a different LLR pattern is adopted compared with a typical LRR in the structure, and in LLR9, located at the C-terminus of this partial ECD structure.

With an available crystal structure of hCG (Lapthorn et al., 1994; Wu et al., 1994; Tegoni et al., 1999), a reasonable working model of the LHR-ECD, coupled with considerable experimental structure-function data, primarily from site-directed mutagenesis, on both the hormone and receptor (Puett and Narayan, 2000; Zeng et al., 2001; Song et al., 2001a,b; Fanelli and Puett, 2002; Vischer et al., 2003a,b; Smits et al., 2003; Vassart et al., 2004), an attempt was made to generate plausible structures for the hCG-LHR-ECD complex. The 3D DOCK program (Gabb et al., 1997) was utilized for rigid-body docking; the structures were then ranked by an evaluation based on electrostatic interactions and geometrical fit, and finally the structures were filtered using several criteria for distance restraints, e.g. results from engineered mutagenesis and known glycosylation sites. While accurate models for the complex cannot be expected from this approach, particularly if binding-induced conformational changes occur in either hCG and/or the ECD, some useful insights may nonetheless be forthcoming.

Figs. 5A and B show predicted complexes for hCG-LHR-ECD and FSH-FSHR-ECD, respectively. The predicted binding interface between hCG and the LHR-ECD is quite extensive and involves both subunits, e.g. loop 2 of α and of β , loop 3 of α , and the seatbelt

loop of β , and numerous LRRs on the receptor. Similar conclusions can be made for the predicted FSH-FSHR complex. A comparison of the predicted and experimentally determined FSH-FSHR-ECD complex shows some areas of agreement and several of considerable disagreement, e.g. the significant conformational change of the α -subunit C-terminal region upon FSHR binding (rotated $\sim 180^\circ$) is of course not predictable in rigid-body docking. Thus, computational approaches such as this can provide only suggestions of complex structures; however, when coupled with good experimental structure-function and chemical data, improbable structures can be discarded with some degree of confidence.

4. Modeling of Other Representative Members of the GPHR Cluster

As mentioned earlier, LHR and FSHR belong the GPHR cluster within the rhodopsin family of GPCRs, a cluster that contains three subgroups or classes (Fredriksson et al., 2003). One class (A) encompasses LHR, FSHR, and TSHR; another class (B) includes three orphan receptors; and the third class (C) contains two GPCRs defined as “relaxin binding” receptors (Hsu, 2003). The GPCRs in this cluster are characterized by relatively large ectodomains with multiple LRRs and some with N-terminal and C-terminal cysteine-rich regions. Interestingly, an analysis of several genomes indicated that these three classes evolved before the evolution of modern insects and vertebrates (Hsu, 2003). BLAST (v.2.2.9) searching (Altschul et al., 1997) and PROSPECT 2.0 threading (Xu and Xu, 2000) identified the best templates to comparatively model the sequence motifs of the ECDs of the three class B and two class C receptors in the GPHR cluster. The sequence alignments derived from the two software programs were combined manually to produce the best templates for comparative modeling using the MODELLER (6v2) software program (Sali et al., 1995). The ECD of the Nogo receptor (He et al., 2003) and of glycoprotein Iba (Huizinga et al., 2002) were used as templates for all five class B and C receptors. The orphan receptors (class B) contain almost twice the number of LRRs as the receptors in classes A and C, and therefore the template chosen to model this larger global structure of the class B receptors was the LRR-containing portion of the *Listeria monocytogenes* interalin A protein (Schubert et al., 2002). The relaxin-binding receptors exhibit one low-density lipoprotein (LDL) receptor “class A” sequence motif in their N-terminal domains that is absent in the other members of the GPHR cluster (Hsu et al., 2000), although a related snail (*Lymnaea stagnalis*) LRR-containing receptor has many LDL receptor “class A” motifs (Tensen et al., 1994). This sequence motif was modeled using an LDL receptor “class A” motif from the crystal structure of the human LDL receptor (Rudenko et al., 2002). Representative models, shown in Fig. 6A and B, represent the first for these portions of the class B and C receptors in the GPHR cluster and may provide insight into the mechanisms by which the LRR motifs of these receptors differentially bind ligands; in time, it may also be possible to predict potential ligands from a knowledge of the binding surface.

5. Mode of Communication of the hCG-LHR-ECD Complex with the Transmembrane Portion of LHR

The so-called “hinge region” of the class A GPHRs refers to the region between the C-terminal end of the LRRs and the entry of the polypeptide chain into the plasma membrane, i.e. helix 1. Since most of this region was deleted in the N-terminal FSHR-ECD fragment used to solve the crystal structure of the FSH-FSHR-ECD complex (Fan and Hendrickson, 2005a), the only suggested structures available are derived from homology modeling (cf. Fig. 3A). The importance of this portion of the LHR-ECD is underscored by the numerous engineered and naturally occurring gain-of-function and loss-of-function mutations that have been described (Bhowmick et al., 1999; Song et al., 2001a,b; Zeng et al., 2001; Vischer et al., 2003a,b; Smits et al., 2003; Nakabayashi et al., 2003).

There are multiple models available suggesting modes of interaction of the hCG-LHR-ECD complex with the transmembrane region (cf. Moyle et al., 1995, 2004, 2005; Fan and Hendrickson, 2005a,b; Karges et al. 2005 for the most recent models); however, to date there is no general consensus on this important aspect of receptor activation. Indeed, controversy remains over whether the ECD restrains the transmembrane portion of LHR in an inactive form, a restraint that is removed concomitant with ligand binding, or whether the transmembrane portion remains in an inactive state unless activated by the hCG-ECD complex. The recent study by Karges et al. (2005) indicates a persistence of tight interactions between the ECD and transdomain portion during LHR activation. The structure presented in Fig. 1 is pictorial only and is not based on any particular set of data. This aspect of hormone-receptor interaction and receptor activation remains a challenge that needs more definitive experimental data.

6. Activation of the Transmembrane Region of LHR

The only GPCR transmembrane structure available is that of rhodopsin in the inactive form (Palczewski et al., 2000), and, despite a plethora of informative biophysical studies on rhodopsin (Farrens, 1996; Dunham and Farrens, 1999; Meng and Bourne, 2001; Choi et al., 2002; Yan et al., 2003), there remains a paucity of information of the exact nature of the intramolecular changes accompanying receptor activation. In the past several years, a great deal has been learned from modeling the endodomain of LHR using *ab initio* and, more recently, comparative modeling approaches (Fanelli, 2000; Fanelli et al., 2001, 2004; Fanelli and Puett, 2002; Fanelli and De Benedetti, 2005; Angelova et al., 2002; Ascoli et al., 2002; Zhang et al., 2005).

With a rhodopsin-based template, the numerous engineered and naturally occurring gain-of-function mutants have been systematically analyzed using molecular dynamic simulations. These results have provided considerable insight into the nature of the hydrogen-bonded network involving conserved residues in the cytoplasmic halves, particularly of helices 1–3, 6, and 7, as well as charge-reinforced hydrogen binding interactions involving the cytosolic extensions of helices 3 and 6, e.g. R464 interacting with both E463 and D564 (Fig. 7A). These interactions contribute to the stability of the inactivate form of LHR. Comparison of this inactive structure with those of a myriad of gain-of-function mutants yielded a parameter, the solvent-accessible surface area (SAS), computed over R464, T467, I468, and K563, that is indicative and predictive of whether any given mutant in the transmembrane region is constitutively activating or not. SAS values greater than and less than 50 Å² correlate with constitutively active (Fig. 7B) and non-constitutively active LHR (Fig. 7A), respectively (Angelova et al., 2002; Fanelli, et al., 2004; Zhang et al., 2005). The higher SAS values are hypothesized to correspond to an open form of LHR that facilitates productive Gsα binding.

With a significant number of characterized engineered and naturally occurring gain-of-function mutation now available (Huhtaniemi and Themmen, 2005), it is interesting to note the variability that exists in function (Fanelli et al., 2001, 2004; Angelova et al., 2002; Ascoli et al., 2002; Zhang et al., 2005). Some, for example, are marginally active, others highly so; some are responsive to exogenous hCG, while others are not (Fig. 8). These and additional observations suggest that there is not a single active state of the receptor in mutation-induced constitutive activation, but rather multiple active conformations, a view consistent with the Ensemble Theory (Onaran and Costa, 1997; Onaran et al., 2000; Kenakin, 2002). The relationship between hormone-induced and mutation-induced LHR activation is an interesting and major problem remaining to be elucidated.

7. Mode of Activated LHR Coupling to G α

As elegantly reviewed by Maudsley et al. (2005), the earlier concept of a “simple linear model” describing ligand binding, receptor activation, G protein binding, GTP exchange for GDP on the α subunit, dissociation of GTP- α from $\beta\gamma$, and subsequent downstream signaling events is no longer valid, and one must now consider multistate models, receptor dimerization, and other factors for a more complete description of GPCR-mediated signaling. For LHR, it is known that both cAMP and IP₃ signaling pathways can be observed (Gilchrist et al., 1996; Ascoli et al., 2002), but the exact role of different G α s and of $\beta\gamma$ are yet to be determined.

Structural studies performed to date have focused on transducin (Gt), involved in vertebrate vision (reviewed in Coleman and Sprang, 1996; Bohm et al., 1997), and on Gi (reviewed in Coleman and Spring, 1996; Bohm et al., 1997) and Gs (Sunahara et al., 1997), respectively, involved in hormone-regulated inhibition and activation of adenylyl cyclase. The available structures can be used as working models to begin combined *in silico* and experimental studies aimed at elucidating LHR-Gs coupling (cf. Fig. 1). In view of the importance of intracellular loops 2 and 3 in G protein coupling of LHR (Fernandez and Puett, 1997; Schulz et al., 1999), as well as FSHR (Timossi et al., 2002), a combined experimental and computational approach has been taken to elucidate the binding contact sites and mode of G protein activation. Site-directed mutagenesis was used to perform Ala-scanning of loop 2 and much of loop 3, followed by characterization of the wild type (WT) and mutant LHRs in transiently transfected HEK 293 cells. Extensive binding and signaling measurements permit determination of the coupling efficiency, Q , that relates receptor expression ($B_{\max}$), hCG binding (K_d or IC_{50}), and cAMP signaling (EC_{50} and maximal response to a saturating dose of hCG, $R_{\max}$) (Ballesteros et al., 1998).

$$Q = 0.5 \{[(K_d + EC_{50}) / (EC_{50})] / (R_{\max} / B_{\max})\}$$

Normalizing the Q values for each mutant, Q_m , to that of WT LHR, $Q_n = Q_m/Q_{wt}$, permits an assessment of coupling efficiency. Since mutations in intracellular loops 2 and 3 have little, if any, impact on hCG binding, Q_n serves to define mutations that, relative to WT LHR, increase cAMP production ($Q_n > 1.0$), are like that of WT receptor ($Q_n = 1.0$), or decrease productive binding and activation of Gs ($Q_n < 1.0$).

Two residues in intracellular loop 2, when substituted with Ala, yielded Q_n values greater than 1.0, W465 and K477, indicating that these side chains are somewhat inhibitory to hCG-mediated signaling. Several residues in loop 2 gave Q_n values less than 0.5, i.e. indicating that the side chains in WT LHR are intimately involved in productive G protein binding and activation. Most of the residues in loop 2 and those studied in loop 3 exhibited Q_n values between 0.5 – 0.8 (marginally involved in coupling) or between 0.8 – 1.2 (comparable to WT LHR within experimental error). The important amino acid residues identified are given in Table 1; interestingly, no comparable residues, i.e. with Q_n less than 0.5, were identified in intracellular loop 3, although Ala-scanning is not yet complete of that region. D564 replacements, as expected (Kosugi et al., 1998; Schulz et al., 1999), gave constitutive activation with replacements other than Glu. Other experimental approaches are now being taken to investigate LHR-G α coupling, and these will be incorporated into molecular dynamics simulations and docking studies to obtain a better understanding of this important aspect of LHR signaling. It is reassuring that the single parameter, SAS, for mutation-induced gain-of-function LHR mutants, that relies on a restricted number of amino acid residues in the cytosolic extensions of helices 3 and 6, is validated by these experimental studies of ligand-induced LHR activation based on Ala-scanning of intracellular loops 2 and 3.

7. Conclusions and Future Directions

Fig. 1 illustrates the general problem to be solved in hormone-mediated LHR activation and subsequent G protein coupling. Structural data are badly needed in many instances, although the availability of structures of related proteins, coupled with the advances in computational biology, enables, in some cases, comparative modeling to be performed with reasonable confidence in the outcome. The wealth of experimental structure-function data that is available and being collected will, nonetheless, provide meaningful limits on potential interaction schemes, but to date there is no adequate mechanism to deal *a priori* with conformational changes associated with interacting systems.

Of the various interactions shown in Fig. 1, e.g. hCG binding to the LHR-ECD and LHR transmembrane binding to Gs, and the implied conformational changes, e.g. hCG-LHR-ECD triggering of receptor activation, transmembrane domain changes, and activation of Gs (binding, GTP-GDP exchange on α , and dissociation of GTP- α from $\beta\gamma$), much remains to be elucidated. While a great deal is known about the mode of hCG binding to the LHR-ECD, there is still much to be learned in terms of specific contact sites; the molecular mechanism by which the hCG-LHR-ECD complex activates the LHR transmembrane region begs to be clarified; the nature of hormone-mediated LHR activation of the transmembrane helices and loops needs to be determined and compared with the working model for mutation-induced receptor activation; and, lastly, with virtually nothing being known at the molecular level on LHR-Gs binding and activation, this important area deserves serious attention. Considerable strides have been made since elucidation of the molecular structures of gonadotropins and since LHR was established to be a GPCR; yet, some of the major questions regarding structure, structure-function relationships, and signaling remain to be adequately answered. The next decade should witness many major contributions to overcome these deficits in our knowledge of this important system in reproductive physiology and pathophysiology.

Acknowledgements

This research was supported by NIH grants DK033973 and DK069711. We extend our gratitude to Dr. Prema Narayan for her interest and overall contributions to this work, and a special thanks to Ms. Judy Gray for expert technical assistance.

References

- Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucl Acids Res* 1997;25:3389–3402. [PubMed: 9254694]
- Angelova K, Fanelli F, Puett D. A model for constitutive lutropin receptor activation based on molecular simulation and engineered mutations in transmembrane helices 6 and 7. *J Biol Chem* 2002;277:32202–32213. [PubMed: 12070159]
- Ascoli M, Fanelli F, Segaloff DL. The lutropin/choriogonadotropin receptor, a 2002 perspective. *Endocrine Rev* 2002;23:141–174. [PubMed: 11943741]
- Ballesteros J, Kitanovic S, Guarnieri F, Davies P, Fromme BJ, Konvicka K, Chi L, Millar RP, Davidson JS, Weinstein H, Sealfon SC. Microdomains in G-protein-coupled receptors. *J Biol Chem* 1998;273:10445–10453. [PubMed: 9553103]
- Bhowmick N, Huang J, Puett D, Isaacs NW, Laphorn AJ. Determination of residues important in hormone binding to the extracellular domain of the luteinizing hormone/chorionic gonadotropin receptor by site-directed mutagenesis and modeling. *Mol Endocrinol* 1996;10:1147–1159. [PubMed: 8885249]
- Bhowmick N, Narayan P, Puett D. Identification of ionizable amino acid residues on the extracellular domain of the lutropin receptor involved in ligand binding. *Endocrinology* 1999;140:4558–4563. [PubMed: 10499511]

- Bohm A, Gaudet R, Sigler PB. Structural aspects of heterotrimeric G-protein signaling. *Curr Opin Biotechnol* 1997;8:480–487. [PubMed: 9265729]
- Chandonia JM, Hon G, Walker NS, Lo Conte L, Koehl P, Levitt M, Brenner SE. The ASTRAL Compendium in 2004. *Nucleic Acids Res* 2004;32:D189–D192. [PubMed: 14681391]
- Choi G, Landin J, Galan JF, Birge RR, Albert AD, Yeagle PL. Structural aspects of metarhodopsin II, the activated form of the G-protein coupled receptor, rhodopsin. *Biochemistry* 2002;41:7318–7324. [PubMed: 12044163]
- Coleman DE, Sprang SR. How G proteins works: a continuing story. *Trends Biochem Sci* 1996;21:41–44. [PubMed: 8851656]
- Couture L, Naharisoa H, Grebert D, Remy JJ, Pajot-Augy E, Bozon V, Haertle T, Salesse R. *J Mol Endocrinol* 1996;16:15–25. [PubMed: 8672229]
- Dunham TD, Farrens DL. Conformational changes in rhodopsin. Movement of helix f detected by site-specific chemical labeling and fluorescence spectroscopy. *J Biol Chem* 1999;274:1683–1690. [PubMed: 9880548]
- Fan QR, Hendrickson WA. Structure of human follicle-stimulating hormone in complex with its receptor. *Nature* 2005a;433:269–277. [PubMed: 15662415]
- Fan QR, Hendrickson WA. Structural biology of glycoprotein hormones and their receptors. *Endocrine* 2005b;26:179–188. [PubMed: 16034172]
- Fanelli F. Theoretical study on mutation-induced activation of the luteinizing hormone receptor. *J Mol Biol* 2000;296:1333–1351. [PubMed: 10698637]
- Fanelli F, Puett D. Structural aspects of luteinizing hormone receptor. *Endocrine* 2002;18:285–293. [PubMed: 12450321]
- Fanelli F, De Benedetti PG. Computational modeling approaches to structure-function analysis of G protein-coupled receptors. *Chem Rev* 2005;105:3297–3351. [PubMed: 16159154]
- Fanelli F, Themmen APN, Puett D. Lutropin receptor function: insights from natural, engineered, and computer-simulated mutations. *IUBMB Life* 2001;51:149–155. [PubMed: 11547916]
- Fanelli F, Verhoef-Post M, Timmerman M, Zeilemaker A, Martens JWM, Themmen APN. Insight into mutation-induced activation of the luteinizing hormone receptor: molecular simulations predict the functional behavior of engineered mutants at M398. *Mol Endocrinol* 2004;18:1499–1508. [PubMed: 15016840]
- Farrens DL, Altenbach C, Yang K, Hubbel WL, Khorana HG. Requirement of rigid-body motion of transmembrane helices for light activation of rhodopsin. *Science* 1996;274:768–770. [PubMed: 8864113]
- Fernandez LM, Puett D. Evidence for an important functional role of intracellular loop II of the lutropin receptor. *Mol Cell Endocrinol* 1997;128:161–169. [PubMed: 9140087]
- Fiser A, Sali A. Modeller: generation and refinement of homology-based protein structure models. *Methods Enzymol* 2003;374:461–491. [PubMed: 14696385]
- Fox KM, Dias JA, Van Roey P. Three-dimensional structure of human follicle stimulating hormone. *Mol Endocrinol* 2001;15:378–389. [PubMed: 11222739]
- Fredriksson R, Lagerström MC, Lundin L-G, Schiöth HB. The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Mol Pharmacol* 2003;63:1256–1272. [PubMed: 12761335]
- Gabb HA, Jackson RM, Sternberg MJ. Modelling protein docking using shape complementarity, electrostatics and biochemical information. *J Mol Biol* 1997;272:106–120. [PubMed: 9299341]
- Gilchrist RL, Ryu KS, Ji I, Ji T. The luteinizing hormone/choriogonadotropin receptor has distinct transmembrane conductors for cAMP and inositol phosphate signals. *J Biol Chem* 1996;271:19283–19287. [PubMed: 8702611]
- He XL, Bazan JF, McDermott G, Park JB, Wang K, Tessier-Lavigne M, He Z, Garcia KC. Structure of the Nogo receptor ectodomain: a recognition module implicated in myelin inhibition. *Neuron* 2003;38:177–185. [PubMed: 12718853]
- Hearn MTW, Gomme PT. Molecular architecture and biorecognition processes of the cystine knot protein superfamily: Part I. The glycoprotein hormones. *J Mol Recog* 2000;13:223–278.

- Huhtaniemi IT, Themmen APN. Mutations in human gonadotropin and gonadotropin-receptor genes. *Endocrine* 2005;26:207–217. [PubMed: 16034174]
- Hsu SYT. New insights into the evolution of the relaxin-LGR signaling system. *Trends Endocrinol Metab* 2003;14:303–309. [PubMed: 12946872]
- Hsu SY, Kudo M, Chen T, Nakabayashi K, Bhalla A, van der Spek PJ, van Duin M, Hsueh AJW. The three subfamilies of leucine-rich repeat-containing G protein-coupled receptors (LGR): Identification of LGR6 and LGR7 and the signaling mechanism for LGR7. *Mol Endocrinol* 2000;14:1257–1271. [PubMed: 10935549]
- Huizinga EG, Tsuji S, Romijn RAP, Schiphorst ME, de Groot PG, Sixma JJ, Gros P. Structures of glycoprotein Iba and its complex with von Willebrand Factor A1 domain. *Science* 2002;297:1176–1179. [PubMed: 12183630]
- Jiang X, Dreano M, Buckler DR, Cheng S, Ythier A, Wu H, Hendrickson WA, El Tayar N. Structural predictions for the ligand-binding regions of glycoprotein hormone receptors and the nature of hormone-receptor interactions. *Structure* 1995;3:1341–1353. [PubMed: 8747461]
- Kajava AV, Vassart G, Wodak SJ. Modeling of the three-dimensional structure of proteins with the typical leucine-rich repeats. *Structure* 1995;3:867–877. [PubMed: 8535781]
- Karges B, Gidenne S, Aumas C, Haddad F, Kelly PA, Milgrom E, de Roux N. Zero-length cross-linking reveals that tight interactions between the extracellular and transmembrane domains of the luteinizing hormone receptor persist during receptor activation. *Mol Endocrinol* 2005;19:2086–2098. [PubMed: 15878956]
- Kenakin T. Efficacy at G-protein coupled receptors. *Nat Rev Drug Discov* 2002;1:103–110. [PubMed: 12120091]
- Kim D, Xu D, Guo JT, Ellrott K, Xu Y. PROSPECT II: protein structure prediction program for genome-scale applications. *Protein Engr* 2003;16:641–650.
- Kobe B, Disenhofer J. Crystal structure of porcine ribonuclease inhibitor, a protein with leucine-rich repeats. *Nature* 1993;366:751–756. [PubMed: 8264799]
- Kosugi S, Mori T, Shenker A. An anionic residue at position 564 is important for maintaining the inactive conformation of the human lutropin/choriogonadotropin receptor. *Mol Pharmacol* 1998;53:894–901. [PubMed: 9584216]
- Kraulis PJ. MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures. *J Appl Crystallog* 1991;24:946–950.
- Laphorn AJ, Harris DC, Littlejohn A, Lustbader JW, Canfield RE, Machin KJ, Morgan FJ, Isaacs NW. Crystal structure of human chorionic gonadotropin. *Nature* 1994;369:455–461. [PubMed: 8202136]
- Maudsley S, Martin B, Luttrell LM. The origins of diversity and specificity in G protein-coupled receptor signaling. *J Pharmacol Exp Ther* 2005;314:485–494. [PubMed: 15805429]
- Meng EC, Bourne HR. Receptor activation: what does the rhodopsin structure tell us? *Trends Pharmacol Sci* 2001;22:587–593. [PubMed: 11698103]
- Merritt EA, Bacon DJ. Raster3D: photorealistic molecular graphics. *Methods Enzymol* 1997;277:505–524.
- Moyle WR, Campbell RK, Venkateswara Rao SN, Ayad NG, Bernard MP, Han Y, Wang Y. Model of human chorionic gonadotropin and lutropin receptor that explains signal transduction of the glycoprotein hormones. *J Biol Chem* 1995;270:20020–20031. [PubMed: 7650020]
- Moyle WR, Xing Y, Lin W, Cao D, Myers RV, Kerrigan JE, Bernard MD. Model of glycoprotein hormone receptor ligand binding and signaling. *J Biol Chem* 2004;279:44442–44459. [PubMed: 15304493]
- Moyle WR, Lin W, Myers RV, Cao D, Kerrigan JE, Bernard MP. Models of glycoprotein hormone receptor interaction. *Endocrine* 2005;26:189–205. [PubMed: 16034173]
- Nakabayashi K, Kudo M, Hsueh AJW, Mauro T. Activation of the luteinizing hormone receptor in the extracellular domain. *Mol Cell Endocrinol* 2003;202:139–144. [PubMed: 12770743]
- Onaran HO, Costa T. Agonist efficacy and allosteric models of receptor structure. *Ann NY Acad Sci* 1997;812:98–115. [PubMed: 9508759]
- Onaran, HO.; Scheer, A.; Cotecchia, S.; Costa, T. A look into receptor efficacy: From the signaling network of the cell to the intramolecular motion of the receptor. In: Kenakin, T.; Angus, J., editors. *Handbook of Experimental Pharmacology*. Springer; Heidelberg: 2000. 148. p. 217–280.

- Palczewski K, Kumasalea T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I, Teller DC, Okada T, Stenkamp RE, Yamamoto M, Miyano M. Crystal structure of rhodopsin: a G protein-coupled receptor. *Science* 2000;289:739–745. [PubMed: 10926528]
- Puett, D.; Narayan, P. Designer gonadotropins and receptors: the prospect of recombinant technology. In: Adashi, EY., editor. *Ovulation: Evolving Scientific and Clinical Concepts*. Springer-Verlag; New York: 2000. p. 295–307.
- Rudenko G, Henry L, Henderson K, Ichtchenko K, Brown MS, Goldstein JL, Deisenhofer J. Structure of the LDL receptor extracellular domain at endosomal pH. *Science* 2002;298:2353–2358. [PubMed: 12459547]
- Sali A, Potterton L, Yuan F, van Vlijmen H, Karplus M. Evaluation of comparative protein modeling by MODELLER. *Proteins: Struct, Func Gen* 1995;23:318–326.
- Schubert W-D, Urbanke C, Ziehm T, Beier V, Machner MP, Domann E, Wehland J, Chakraborty T, Heinz DW. Structure of internalin, a major invasion protein of *Listeria monocytogenes*, in complex with its human receptor E-cadherin. *Cell* 2002;III:825–836. [PubMed: 12526809]
- Schulz A, Schonenberg T, Paschke R, Schulz G, Gudermann T. Role of the third intracellular loop for the activation of gonadotropin receptors. *Mol Endocrinol* 1999;13:181–190. [PubMed: 9973249]
- Smits G, Campillo M, Govaerts C, Janssens V, Richter C, Vassart G, Pardo L, Costagliola S. Glycoprotein hormone receptor: determinants in leucine-rich repeats responsible for ligand specificity. *EMBO J* 2003;22:2692–2703. [PubMed: 12773385]
- Song YS, Ji T, Beauchamp J, Isaacs NW, Ji TH. Hormone interactions to Leu-rich repeats in the gonadotropin receptors. I Analysis of Leu-rich repeats of human luteinizing hormone/chorionic gonadotropin receptor and follicle-stimulating hormone receptor. *J Biol Chem* 2001a;276:3426–3435. [PubMed: 10880516]
- Song YS, Beauchamp J, Isaacs NW, Ji TH. Hormone interactions to Leu-rich repeats in the gonadotropin receptors. II Analysis of Leu-rich repeat 4 of human luteinizing hormone/chorionic gonadotropin receptor. *J Biol Chem* 2001b;276:3436–3442. [PubMed: 10884391]
- Sunahara RK, Tesmer JJ, Gilman AG, Sprang SR. Crystal structure of the adenylyl cyclase activator G α . *Science* 1997;278:1943–1947. [PubMed: 9395396]
- Takeda S, Kadowaki S, Haga T, Takaesu H, Mitaku S. Identification of G protein-coupled receptor genes from the human genome sequence. *FEBS Lett* 2002;520:97–101. [PubMed: 12044878]
- Tegoni M, Spinelli S, Verhoeyen M, Davis P, Cambillau C. Crystal structure of a ternary complex between human chorionic gonadotropin (hCG) and two Fv fragments specific for the α and β -subunits. *J Mol Biol* 1999;289:1375–1385. [PubMed: 10373373]
- Tensen CP, Kesteren ERV, Planta RJ, Cox KJA, Burke JF, van Heerikhuizen H, Vreugdenhil E. A G protein-coupled receptor with low density lipoprotein-binding motifs suggests a role for lipoproteins in G-linked signal transduction. *Proc Natl Acad Sci USA* 1994;91:4816–4820. [PubMed: 8197140]
- Timossi C, Maldonado D, Vizcaino A, Lindau-Shepard B, Conn PM, Ulloa-Aguirre A. Structural determinants in the second intracellular loop of the human follicle-stimulating hormone receptor are involved in G α s protein activation. *Mol Cell Endocrinol* 2002;189:157–168. [PubMed: 12039074]
- Vassart G, Pardo L, Costagliola S. A molecular dissection of the glycoprotein hormone receptors. *Trends Biochem Sci* 2004;29:119–126. [PubMed: 15003269]
- Vischer HF, Granneman JCM, Bogerd J. Opposite contribution of two ligand-selective determinants in the N-terminal hormone-binding exodomain of human gonadotropin receptors. *Mol Endocrinol* 2003a;17:1972–1981. [PubMed: 12869592]
- Vischer HF, Granneman JCM, Noordam MJ, Mosselman S, Bogerd J. Ligand selectivity of gonadotropin receptors. Role of the β -strands of extracellular leucine-rich repeats 3 and 6 of the human luteinizing hormone receptor. *J Biol Chem* 2003b;278:15505–15513. [PubMed: 12598521]
- Wu H, Lustbader JW, Liu Y, Canfield RE, Hendrickson WA. Structure of human chorionic gonadotropin at 2.6Å resolution from MAD analysis of the selenomethionyl protein. *Structure* 1994;2:545–558. [PubMed: 7922031]
- Xu Y, Xu D. Protein threading using PROSPECT: design and evaluation. *Proteins: Struct, Func Gen* 2000;40:343–354.

- Yan ECY, Kazmi MA, Ganim Z, Hou JM, Pan D, Chang BSW, Sakmar TP, Mathies RA. Retinal counterion switch in the photoactivation of the G protein-coupled receptor rhodopsin. *Proc Natl Acad Sci USA* 2003;100:9262–9267. [PubMed: 12835420]
- Zeng H, Phang T, Song YS, Ji I, Ji T. The role of the hinge region of the luteinizing hormone receptor in hormone interaction and signal generation. *J Biol Chem* 2001;276:3451–3458. [PubMed: 10964934]
- Zhang M, Mizrahi D, Fanelli F, Segaloff DL. The formation of a salt bridge between helices 3 and 6 is responsible for the constitutive activating and lack of hormone responsiveness of the naturally occurring L457R mutation of the human lutropin receptor. *J Biol Chem* 2005;280:26169–26176. [PubMed: 15908694]

Abbreviations

LH	luteinizing hormone
LHR	LH receptor
CG	chorionic gonadotropin
GPCR	G protein-coupled receptor
GPHR	glycoprotein hormone receptor
FSH	follicle stimulating hormone
FSHR	FSH receptor
TSH	thyroid stimulating hormone
TSHR	TSH receptor
LRR	leucine-rich repeat
ECD	ectodomain
hCG	human CG
LDL	low density lipoprotein
HEK	human embryonic kidney
Q	coupling efficiency

WT

wild type

Fig. 1.

Schematic representation of the hCG-LHR-Gs protein complex. This illustration depicts a complex containing six distinct proteins, the heterodimeric hormone, hCG (with one α and one β subunit, colored in yellow and orange, respectively), bound to the LHR-ECD (visualized in pink) on the extracellular face of the plasma membrane and the transmembrane portion of LHR (helices colored green) bound to the heterotrimeric G protein (with one each of α , β , and γ subunits, represented in royal blue, light blue, and red, respectively; these α and β subunits are unrelated to those comprising hCG) on the intracellular face of the plasma membrane. As discussed in the text, the structure shown for hCG is based on crystallographic data, that for LHR on homology modeling, and that for $G\alpha$ is actually the crystal structure of the homologous

$G\alpha_t$ subunit complexed with $\beta\gamma$. The N-linked oligosaccharides on hCG and the LRR domain of the LHR ECD were modeled as biantennary complex forms (except that on $\alpha N52$ as a hybrid form) and presented in a ball-and-stick representation. While there is some confidence that the hCG-LHR-ECD complex is reasonably accurate, the positioning of this complex with the transmembrane portion of LHR is simply pictorial, as is the interaction surface of LHR and the G protein. This figure was generated with the molecular visualization programs, MOLSCRIPT (Kraulis, 1991) and Raster3D (Merritt and Bacon, 1997).

Fig. 2.
Structure of a typical LHR in the LHR-ECD. The amino acid sequences of LxxLxLxxNxxLxxLPxxaFxxLxx (see text for definition) adopts a structure in which the xxLxLxx forms a β -strand and the remainder adopts a loop structure, with the side chains of the conserved L, N, and F residues pointing inside to form a hydrophobic core.

Fig. 3. Predicted structure of the LHR-ECD. A. Based on homology modeling with the structures of the Nogo receptor (He et al., 2003) and glycoprotein Iba α (Huizinga et al., 2002) as outlined in the text. B. A large N-terminal fragment (residues 1 – 241) of the LHR-ECD based on homology modeling with the structure of the FSH-ECD. C. Comparison of the models of the LHR-ECD N-terminal fragment based on homology modeling with the Nogo receptor/glycoprotein Iba α and the FSH-ECD N-terminal fragment. The structures were drawn with MOLSCRIPT (Kraulis, 1991).

Fig. 4. Predicted and experimental structures of the FSHR-ECD N-terminal fragment (residues 1 – 241). A. Molecular modeling with the Nogo receptor (He et al., 2003) and glycoprotein Iba (Huizinga et al., 2002) as templates. B. Crystal structure shown without the bound FSH (Fan and Hendrickson, 2005a). C. Comparison of the predicted structure of FSH-ECD 1 – 241 (from A) with that of the crystal structure (from B). The structures were drawn with MOLSCRIPT (Kraulis, 1991).

Fig. 5.

Predicted and experimental structures of gonadotropin-receptor-ECD complexes. A. Predicted structure of the hCG-LHR-ECD complex using rigid-body docking. B. Predicted structure of the FSH-FSHR-ECD complex using rigid-body docking. C. Comparison of the predicted and experimentally determined (Fan and Hendrickson, 2005a) structure of the FSH-FSHR-ECD complex.

Fig. 6. Predicted structures for extracellular motifs of representative members of class B and class C GPHRs. A. The orphan receptor, hLGR4, contains about twice the number of LRRs as other members of the GPHR cluster. B. The relaxin-binding receptor, hLGR8, contains multiple LRRs and one LDL receptor “class A” motif. These figures were generated with the molecular visualization programs MOLSCRIPT (Kraulis, 1991) and Raster3D (Merritt and Bacon, 1997).

Fig. 7.

Average minimized structures of the WT LHR (A) and of the D564G constitutively active mutant (B). Only the cytosolic halves of helices 3 and 6 are shown, seen in a direction parallel to the putative membrane surface and colored in green and cyan, respectively. The side chains of E463, R464, and D564 are also shown, represented by licorice models and colored according to their polarity (i.e. blue for cations and red for anions). The SAS computed over R464, T467, I468, and K463 is represented by gray dots. This index is $28 \text{ \AA}^2$ for WT LHR and $120 \text{ \AA}^2$ for the D564G mutant (Fanelli et al., 2004).

Fig. 8. Comparison of WT LHR and gain-of-function LHR mutants in response to exogenous hCG. WT LHR and constitutively activating mutants were expressed in HEK 293 cells, and the intracellular concentrations of cAMP were determined in the absence and presence of a saturating concentration of hCG (with an added inhibitor of cyclic nucleotide phosphodiesterase).

Table 1

Summary of signaling characteristics of amino acid residues in intracellular loop 2 of LHR that are important in G protein coupling^a

LHR (n)	B _{max} (% WT)	EC ₅₀ (nM)	R _{max} (%)	Q _n
WT (21)	100.0	0.29 ± 0.05	100.0	1.00
mutant:				
I460A (4)	43 ± 10	0.32 ± 0.05	47 ± 12	0.41 ± 0.00 ³
T461A (3)	108 ± 26	0.26 ± 0.03	51 ± 12	0.30 ± 0.02
R464A (8)	6 ± 1	2.47 ± 0.43	4.6 ± 1.9	0.10 ± 0.01
I468A (5)	19 ± 9	1.00 ± 0.34	4.5 ± 1.7	0.10 ± 0.01
I472A (4)	187 ± 54	0.41 ± 0.09	91 ± 27	0.34 ± 0.04
I478A (3)	108 ± 18	0.74 ± 0.11	54 ± 5	0.32 ± 0.01

^aWT and mutant LHRs were expressed in HEK 293 cells, and binding studies (not shown) were conducted with [¹²⁵I]-hCG in competitive and saturation isotherms. IC₅₀s ranged from 0.3 – 1.4 nM, and the expression level (B_{max}) of WT LHR was 29.0 ± 8.5 fmol/ml. Basal cAMP values were the same between WT and mutant LHRs, and R_{max} denotes the maximal cAMP produced in response to a saturating dose of hCG, normalized to that of WT LHR (100%). The coupling efficiency, Q_n, is defined in the text and normalized to that of WT LHR (Q = 1.00). All Q_n values for the six mutants are significantly different from that of WT LHR (P<0.05).