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The luteinizing hormone receptor: Insights into structure-function relationships and hormone-receptor-mediated changes in gene expression in ovarian cancer cells

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Abstract

The luteinizing hormone receptor (LHR), one of the three glycoprotein hormone receptors, is necessary for critical reproductive processes, including gonadal steroidogenesis, oocyte maturation and ovulation, and male sex differentiation. Moreover, it has been postulated to contribute to certain neoplasms, particularly ovarian cancer. A member of the G protein-coupled receptor family, LHR contains a relatively large extracellular domain responsible for high affinity hormone binding; transmembrane activation then leads to G protein coupling and subsequent second messenger production. This review deals with recent advances in our understanding of LHR structure and structure-function relationships, as well as hormone-mediated changes in gene expression in ovarian cancer cells expressing LHR. Suggestions are also made for critical gaps that need to be filled as the field advances, including determination of the three-dimensional structure of inactive and active receptor, elucidation of the mechanism by which hormone binding to the extracellular domain triggers the activation of Gs, clarification of the putative roles of LHR in non-gonadal tissues, and the role, if any, of activated receptor in the development or progression of ovarian cancer.

Keywords

LH receptor; Gonadotropin; Human chorionic gonadotropin; Luteinizing hormone; Ovarian cancer; SKOV3 cells

1. Introduction

The glycoprotein hormone receptors (GpHRs) constitute a sub-family of the large G protein-coupled receptor (GPCR) family. Three receptors form the GpHRs, the luteinizing hormone (LH) receptor (LHR), the follicle-stimulating hormone (FSH) receptor (FSHR), and the thyroid-stimulating hormone (TSH) receptor (TSHR) (Ascoli et al., 2002; Caltabiano et al., 2008; Ascoli and Puett, 2009; Kleinau and Krause, 2009). FSHR and TSHR bind single

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glycoprotein hormones, FSH and TSH, respectively; LHR, on the other hand, binds two glycoprotein hormones, LH and human chorionic gonadotropin (hCG). Each of the four heterodimeric glycoprotein hormones contains a common α subunit and a hormone-specific β subunit. While there are single genes encoding LHβ, FSHβ, and TSHβ, there are six *hcgβ* genes, these being clustered with *lhβ* on chromosome 19 in the human genome (Ascoli and Puett, 2009).

The glycoprotein hormones and their cognate receptors regulate reproductive and metabolic processes; moreover, LHR activation and subsequent androgen production is required for male sex differentiation. The gonadotropins and their receptors are responsible for gonadal steroidogenesis and ovulation, while TSH and TSHR regulate thyroid hormone production. In addition to their established roles in the regulation of normal reproduction, development, and metabolism, the GpHRs are also implicated in various pathophysiological conditions. For example, mutations in *lhr* are known to be responsible for certain reproductive disorders (Themmen, 2005, Segaloff, 2009), and LH-mediated activation of LHR has been suggested to contribute to the etiology and/or progression of ovarian cancer (Leung and Choi, 2007; Choi et al., 2007; Mandai et al., 2007).

The GpHRs contain two major domains approximately the same size: (a) a relatively large glycosylated N-terminal ectodomain (ECD) containing leucine-rich repeats (LRRs) capped by Cys-rich regions, the latter forming a portion of a hinge region, and (b) a transmembrane domain (TM) with seven membrane spanning helices, three extracellular loops (ecls), three intracellular loops (icls) and a short icl 4, an eighth cytoplasmic helix parallel to the plasma membrane, and a cytoplasmic tail (Ascoli and Puett, 2009). The ECD and TM domains have important and distinct functional roles, namely hormone binding and signal transduction, respectively. Most of the sequential steps involved after hormone binding to the ECD until G protein activation on the inner face of the plasma membrane remain poorly understood. In many experimental systems, LH or hCG binding to LHR results in activation of both protein kinase A and protein kinase C. At relatively low concentrations of LH and hCG, Gsα appears to be the preferred signaling pathway, resulting in a rapid increase in the intracellular concentration of cAMP.

Following the earlier purification and characterization studies of the hormones and receptors at the protein and gene levels, advances in structural biology of these complex glycoprotein hormones and the ECDs of two of the three GpHRs have added a critical new dimension to our understanding of hormone and receptor structure-function relationships. Crystal structures are now available for deglycosylated hCG (Lapthorn et al., 1994; Wu et al., 1994), an antibody-bound glycosylated hCG (Tegoni et al., 1999), and a partially deglycosylated human FSH, both free (Fox et al., 2001) and bound to a large N-terminal fragment of the FSHR ECD (Fan and Hendrickson, 2005, 2007). Moreover, the NMR solution structure of the deglycosylated human α subunit has been determined (De Beer et al., 1996; Erbel et al., 1999). The heterodimeric hormones, members of the cystine-knot growth factor protein family, were found to be highly asymmetric with intertwined subunits forming a large surface area of subunit-subunit contact. A most unusual feature was the presence of a “seatbelt” in hCG and FSH in which an intramolecular disulfide loop in the β subunit (Cys-90-Cys-110 in hCGβ and Cys-84-Cys-104 in FSHβ) is wrapped around a region of the α subunit; contained within this seatbelt is a determinant loop (Cys-93-Cys-100 in hCGβ and Cys-87-Cys-94 in FSHβ) that appears to confer hormone specificity.

Crystal structures are now available for several natural and engineered recombinant GPCRs (see reviews by Mustafi and Palczewski, 2009; Rosenbaum et al., 2009 with references to the original reports). For example, there are several structures of bovine and squid rhodopsin (inactive) and of opsin (an active form of rhodopsin). Structures have also been published

for the turkey β_1-adrenergic receptor, the human β_2-adrenergic receptor, and the human A_{2A} adenosine receptor, all with bound antagonist or inverse agonist. As shown in Fig. 1, the four GPCRs have similar overall structures; the root mean square deviation of the TM regions is < 3 A for these receptors in the inactive state. The similarity in the relative orientations of the TM helices in the GPCRs of known crystallographic structure engenders confidence that these can be used for comparative modeling of the GpHRs. Although not presented in Fig. 1, a comparison of the rhodopsin and opsin structures shows that subtle changes occur in the TM regions, but the most significant changes are at or near the cytoplasmic surface. Here, the cytosolic region of TM6 shifts more than 6 A from the center of the bundle in the inactive state toward TM5. This reorientation involves a break of an ionic "lock" in which the Arg of the (Asp/Glu)Arg(Tyr/Trp) (DRY) motif at the cytoplasmic end of TM3, bound to a Glu in the cytosolic extension of TM6 in rhodopsin, binds to a Tyr on TM5 in opsin. Other alterations at or near the cytoplasmic surface accompany the inactive → active state conformation.

While no structures are available of the intact GpHRs, Fig. 2 shows the crystal structure of a complex of a partially deglycosylated FSH bound to a partially degylcosylated FSHR ECD (amino acid residues 1–268) (Fan and Hendrickson, 2005,2007). Also, a structure is available of a deglycosylated complex of a monoclonal antibody bound to a large N-terminal fragment of the human TSHR ECD (Sanders et al., 2007). These seminal studies have delineated important structural features of the hormones, receptor ECDs, and hormone-receptor complexes.

Of the various gynecological malignancies, ovarian cancer is the most lethal, in part because it is usually diagnosed at a late stage (Choi et al., 2007). Epidemiological studies have shown that infertility and nulliparity are risk factors for ovarian cancer while oral contraceptive use and pregnancy correlate with a lower incidence of ovarian cancer. These and other studies have led to the suggestion that gonadotropins and their receptors may function in some manner to contribute to the etiology of ovarian cancer or to the progression of the disease (Leung and Choi, 2007; Choi et al., 2007; Mandai et al. 2007). Such an effect would be particularly pronounced in postmenopausal women where gonadotropin levels are high. The majority of ovarian cancers are epithelial in nature, arising from the ovarian surface epithelium which has been shown to express LHR and FSHR and to respond to gonadotropins (Choi et al., 2007).

This review focuses on LHR from the perspective of structure-function relationships and its putative role in ovarian cancer. Not intended to be comprehensive, only selected salient findings of the past several years are discussed.

2. Structure and structure-function relationships of LHR

The amino acid sequences of the two human gonadotropin receptors, LHR and FSHR, are shown in Fig. 3, with several important regions denoted. The ECD, encompassing over 330 amino acid residues, contains the N-terminal Cys-rich region, the LRR region, and a hinge region terminating with a Cys-rich sequence. The seven TM helices are depicted, along with the extracellular and intracellular loops, and the eighth (non-TM) helix, the structure being terminated by the C-terminal tail.

Since no structural data are available for LHR, comparative modeling with other LRR proteins has been used to generate working models of the ECD (Bhowmick et al., 1996; Vassart et al., 2004; Bogerd, 2007; Puett et al., 2005, 2007; Caltabiano et al., 2008 and references therein for earlier models). The more recent models are similar to each other, not surprising since all were derived from a small subset of LRR proteins. Two very similar models of the LHR ECD were found (Puett et al., 2007) based on comparative modeling

using the Nogo receptor (He et al., 2003) and glycoprotein Ibα (Huizinga et al., 2002) as templates. These LHR ECD models were very similar to the model obtained when the crystal structure of the truncated FSHR ECD with bound FSH (Fan and Hendrickson, 2005) served as the template; the major differences being in LRRs 5 and 9 (Puett et al., 2007). A working model of hCG bound to the LHR ECD is shown in Fig. 4 and was obtained by rigid body docking using the crystal structureof hCG and the predicted structure of the LHR ECD (Puett et al., 2007).

Also using comparative modeling, proposed structures are available for the TM domain of LHR. Fig. 5 depicts models for LHR in the basal state and when activated by a gain-of-function mutation (Angelova et al., 2002). This model identified a number of intrahelical charge-reinforced H-bonding interactions that maintain the receptor in the inactive state, including the Arg in the (Asp/Glu)Arg(Tyr/Trp) or DRY motif, Arg-464(3.50 in the Ballesteros and Weinstein (1995) numbering scheme) and Asp-564(6.30), located in the cytosolic extensions of TM helices 3 and 6, respectively; Asp-578(6.44) and Asn-615(7.45)/Asn-619(7.49), and others. The solvent accessible surface area (SAS), measured over cytosolic amino acid residues, has proven to be a good indicator of constitutive receptor activation with mutants having SAS values above a threshold exhibiting constitutive activation and those with SAS values below a threshold characterized with basal-type signaling (Zhang et al., 2007;Angelova et al., 2008;Feng et al., 2008;Fanelli et al. 2009).

Numerous naturally occurring gain-of-function and loss-of-functions mutations have been described (Segaloff, 2009); also, single, double, and triple mutants throughout LHR have been made and characterized, as have various chimeric receptors. Two websites on the GpHRs, http://gris.ulb.ac.be/ (Van Durme et al., 2006) and http://www.ssfa-gphr.de/main/ssfa.php (Kleinau et al., 2007), are excellent sources for this vast literature. These studies have provided important information on the nature of the LHR amino acid residues that participate in hormone binding to the ECD, those responsible for maintaining the receptor in an inactive state in the absence of hormone, and those involved in G protein binding and activation. Naturally occurring activating mutations have been described in transmembrane helices 1, 2, 3, 5, and 6, and naturally occurring inactivating mutations have been reported in exons 1, 4–8, 10, and 11 of the ECD, in icls 1 and 3, and in transmembrane helices 1 and 4–7. These and the many hundreds of engineered mutations have provided considerable insight into the hormone-receptor contact sites and residues involved in signal transduction.

A number of critical amino acid residues, many serving as both positive and negative hormone specificity determinants (Moyle et al., 1994), have been identified in the gonadotropin receptor ECDs (Bhowmick et al. 1996, 1999; Song et al., 2001a, b; Smits et al., 2003; Vischer et al., 2003a, b, 2006; Vassart et al., 2004; Zhang et al., 2007; Caltabiano et al., 2008; Feng et al., 2008). It was recently suggested by Caltabiano et al. (2008) that the determinant loop of hCGβ, which contains two positively charged residues, Arg-94 and Arg-95, and one conserved negatively charged residue, Asp-99, interact with LHR as follows: Arg-94 with Glu-206, Arg-95 with Glu-154, and Asp-99 with Asn-107. (The identical residues are also present in LHβ.) The determinant loop in FSHβ, in contrast, contains three negatively charged residues, Asp-88, Asp-90, and Asp-93. Other hCG-LHR interactions proposed in this model are hCGβ Asp-105 with Tyr-58 and hCGβ Lys-104 with Glu-79. Lastly, the hinge region of LHR has been demonstrated to be important in hormone binding and hormone-mediated signaling (Zeng et al., 2001; Bruysters et al., 2008), but its exact function remains elusive.

In a recent study the contributions of individual amino acid residues in the cytosolic extensions of TM helices 3, 5, and 6 and of icls 2 and 3 of the LHR were mapped for

functional significance using Ala-scanning mutagenesis and computational modeling (Angelova et al., 2008). It was found that residues comprising the cytosolic extension of TM3 and the N-terminal portion of icl2 contributed significantly to G protein coupling and activation; in contrast, icl3 contributed very little. Compared to that of wild type LHR, the coupling efficiencies of the Ala mutants in the cytosolic extension of TM3 and icl2 are shown in Fig. 6 (Glu-463-Ala failed to express and residue 471 is a naturally occurring Ala). The coupling efficiency is a parameter that accounts for the level of receptor expression, hormone-receptor binding affinity, the ED_{50} for cAMP production and the maximal cAMP produced at a saturating concentration of hCG (Ballesteros and Weinstein, 1995) as defined in the legend to Fig. 6. A perusal of the data shows that a number of amino acid residues are involved, either directly or indirectly, in G protein coupling and activation. Of the 10 residues in the cytosolic extension of TM3, six appear to be important in productive coupling; not surprisingly, Arg-464 of the DRY motif is one of them. In this study, the Ala replacements had no appreciable effect on the hormone binding affinity, which is not surprising since these are located on an icl. The expression levels did vary somewhat, but the major impact on the coupling efficiencies arose from a significant increase in the ED_{50} and a significant decrease in R_{max}, as found particularly with R464A and I468A. In contrast to the findings documented in Fig. 6, Ala-scanning mutagenesis of the cytosolic extensions of TM5/6 and icl 3 revealed only a few residues that appear to be important in effective LHR-G-protein coupling. Of the many residues evaluated in the cytosolic extensions of TM helices 3, 4, 5, and 6, and of icls 2 and 3, only D564 in the cytosolic extension of TM6 seems to maintain LHR in an inactive state.

Yoked (tethered) forms of hormone and receptor have been prepared in which a single chain hCG, either in the N-α-β-C or N-β-α-C orientation, was fused with the N-terminus of the full-length LHR and expressed and characterized in cultured cells (Wu et al., 1996; Puett et al., 1998; Narayan et al., 2000) and in transgenic mice (Meehan et al., 2005; Meehan and Narayan, 2007; Coonce et al., 2009). The receptor was found to be constitutively active due to the covalently attached hormone, although it is unknown if the hormone binds to and activates the receptor to which it is attached or to a neighboring receptor, i.e. *cis* or *trans* activation (Ji et al., 2002). This engineered version of a constitutively activated LHR complements the studies being done with constitutively active mutants of LHR arising from single base changes in *lhr* (Themmen, 2005; Segaloff, 2009; Ascoli and Puett, 2009). Parallel studies using yoked hCG-FSHR and hCG-TSHR showed that each of the receptors could be activated by hCG, albeit to a small extent with FSHR (Schubert et al., 2003). This model system was also used with yoked α-LHR and yoked hCGβ-LHR to show that the individual subunits are incapable of activating LHR under conditions mimicking high concentrations (Narayan et al., 2002); likewise, yoked versions of minimized forms of hCGβ fused to α-LHR identified minimal responses at best (Schubert and Puett, 2003). This system has also been used to prepare and characterize soluble yoked hCG-LHR ECD C-terminal truncation complexes for circular dichroic spectroscopy in which it was demonstrated that secondary structure is lost as the hinge region is removed (Fralish et al., 2003).

A number of recent studies have addressed the important issue of LHR dimerization and formation of higher order oligomers. Self association of the receptor has been suggested for some time, however considerable controversy existed over whether such association was simply artifactual or not. Different investigators, using a variety of experimental and computational approaches, have within the past few years made a compelling case for LHR dimerization and oligomerization. Using a combination of Western blotting and immunoprecipitation of transiently and stable transformed cells, Tao et al. (2004) reported bands of 67, 84, 166, and 240 kDa, consistent with other reports. The 67 kDa band represents the immature precursor to LHR, and the 84 kDa band corresponds to the mature

cell surface monomeric form. The 166 and 240 kDa bands are probably attributable to dimers and higher order oligomers, although the possible involvement of other, non-LHR proteins has not been excluded. Interestingly, it was found that hCG increased the relative amounts of dimers and higher order structures. Several reports have appeared in which fluorescence photobleaching (Roess et al., 2000) and bioluminescence resonance energy transfer (BRET) (Urizar et al., 2005; Zhang et al., 2009; Guan et al., 2009) experiments were done on wild-type and mutant receptors showing the presence of LHR dimers/oligomers on the cell surface. Complementing these reports, a series of elegant experiments was conducted to demonstrate *trans*-activation of LHR (Ji et al., 2002; Lee et al., 2002; Jeoung et al. 2007). These studies utilized two forms of LHR, one deficient in hormone binding and the other deficient in hormone-mediated signaling. When co-expressed in cells, it was shown that receptor function could be restored. A recent report described the use of transgenic mice co-expressing signaling deficient and binding deficient mutants of LHR to demonstrate the rescue of LHR and restore normal physiological actions of LH (Rivero-Muller et al., 2010). These *in vivo* results argue strongly for LHR association. Lastly, computational studies have suggested that TM4 in particular (as well as TM5 and TM6) is important in mediating LHR dimerization (Fanelli, 2007).

3. LHR and ovarian cancer

A number of ovarian cancer epithelial cell lines are available, some of which have been shown to express the LHR mRNA and some that do not, the results on SKOV-3 cells being controversial. For example, in two studies PCR failed to detect any mRNA; moreover, no functional LHR was present as judged by a lack of both [^{125}I]hCG specific binding and increased DNA synthesis (Parrott et al., 2001; Warrenfeltz et al., 2008). Another study, however, reported the presence of LHR mRNA in SKOV-3 cells and showed an LH-mediated increase in cell invasiveness and effects on mRNA levels of matrix metalloproteinases (MMP-2 and MMP- 9), tissue inhibitor of metalloproteinase (TIMP-1 and TIMP-2), and plasminogen activator inhibitor (Choi et al., 2006). The reason(s) for the discrepancies are unknown, although the study in which a positive effect was reported found effects only at high levels of human LH, 100 and 1,000 ng/mL. In a recent study, stable transformants of SKOV-3 cells were prepared that expressed about 12,000 LH receptors/cell, binding hCG with a K_d of 0.3–0.8 nM (Warrenfeltz et al., 2008). The LHR+ cells exhibited increased cAMP and inositol phosphate levels in response to hormone. cAMP up-regulates ErbB-2 (Her-2) in Schwann cells (Friegen et al. 2005), and over-expression of ErbB-2 in breast, ovarian, and other tumors correlates with a poorer prognosis for the patient (Holbro et al., 2003). Experiments were devised to determine if LH addition to the LHR+ SKOV-3 cells up-regulated ErbB-2 gene expression, resulting in increased concentrations of protein and phosphorylated ErbB-2, i.e. the active form (Warrenfeltz et al., 2008).

Gene expression was measured using purified cellular RNA that was converted to cDNA, followed by a Taqman Low Density Array with 18S RNA as an internal control. Results from this real-time expression array format, after filtering out very low expressing genes ($C_t \leq 34$) and genes with changes between control and experimental samples of < 2-fold, are summarized for selected genes in Fig. 7. The genes were chosen for having been reported to have some association with proliferation, cell adhesion, invasion, or LHR signaling. Panel A compares the LHR+ SKOV-3 cells with mock-transfected (LHR-) cells and shows that several genes are up-regulated, e.g. *BRCA1*, *EGFR*, *IGF2R*, *LHR*, and others, while *IGFBP 5* and *6*, *MMP 1* and *13*, *TIMP3*, and others are down-regulated, presumably due to a slight elevation in the concentrations of the intracellular second messengers or perhaps to a gonadotropin-independent effect of LHR. A time course of the changes in gene expression following addition of LH to the LHR-expressing cells is given in Panel B (Fig. 7). Expression of *ErbB-2*, as postulated, is increased by LH-mediated activation of LHR. In

addition, genes for *LHR*, *MMP25*, *IGFBP 1* and *3*, and others are also up-regulated, while gene expression of *hCGβ*, *TIMP3*, and others are down-regulated. Interestingly, expression of *LHβ* and *hCGβ* are both down-regulated.

Increased levels of ErbB-2 mRNA were shown to correlate with increased levels of protein and phosphorylated protein (Fig. 8). Other studies demonstrated that forskolin and 8-Br-cAMP increased the intracellular protein concentration of ErbB-2 in both mock transfected and LHR-expressing SKOV-3 cells, while inhibitors of protein kinase A, protein kinase C, EGFR, and the kinase activity of ErbB-2 decreased ErbB-2 protein levels in LHR-expressing cells in the presence of LH (Warrenfeltz et al. 2008).

Interestingly, despite the increase in expression of ErbB-2 in LHR+ SKOV-3 cells, cell proliferation, invasiveness, and migration were reduced by LH. This finding is surprising in view of the reports that increased ErbB-2 expression correlates with increase proliferation ((Yu et al., 1993; Hsieh et al., 2000). Others, however, have reported that LH and hCG reduce proliferation of ovarian epithelial cells and tumors, as well as the breast cancer cell line, MCF-7 (Zheng et al., 2000; Tourgeman et al., 2002; Rao et al., 2004). The low density array data by and large support the observation that LH-mediated LHR activation of the ovarian cancer cells decreases invasiveness and migration. For example, with the exception of MMP25, the expression of the other matrix metalloproteinases on the array were down-regulated, as was the protease, PLAU, while several genes encoding cell adhesion and basement membrane proteins, e.g. *COL4A3*, *COL4A4*, *NID2*, *ITGB8*, and *LAMA3*, were up-regulated. The time course over which gene expression was measured shows that, for this limited number of genes, most are appreciably altered after incubation with LH for longer than 30 min; the only early response genes altered 2-fold or more within 30 min were those for *LHR*, *MMP1*, *MMP13*, and *MMP25*.

The literature is rather confusing on the roles of gonadotropins in ovarian and breast cancer (cf. reviews by Leung and Choi, 2007; Choi et al., 2007; Mandai et al., 2007 and earlier studies referenced therein), probably reflecting the heterogeneity of the tumors and the established cell lines being used. The recent results on an engineered LHR+ ovarian epithelial cancer cell line show that LH slightly reduces proliferation, invasiveness, and migration in short term assays in spite of an LH-mediated increase in a protein such as ErbB-2 that is usually associated with increased proliferation. Other studies on a variety of ovarian cancer cell lines have shown, however, that gonadotropins can increase proliferation, migration, and invasiveness. Since many ovarian cancer express LHR and FSHR, this area of investigation requires additional research to sort out the myriad, and at times conflicting, results that have been published.

4. Conclusions

Tremendous gains have been made in our knowledge of LHR at the molecular level and its mode of ligand binding and subsequent activation. Nonetheless, major gaps remain that must be addressed for a better understanding of this important GpHR. The similar structures of the FSHR and TSHR ECDs engenders confidence that that of LHR will not be drastically different; yet, a structure is needed and particularly one with bound hormone to the full length ECD to ensure accuracy and completeness in identifying hormone-receptor contact sites. Elucidation of the structure and function of the hinge region, known to be important in LHR function, is required, as is the mechanism by which hormone binding to the ECD is transmitted via the TM helices resulting in G protein activation (see Ascoli and Puett, 2009; Kleinau and Krause, 2009). In time, as methods for crystallizing membrane proteins become more developed, it is anticipated that structures will become available for full-length LHR with and without bound hormone. Until such time, however, much can be done to elucidate

the many areas that are poorly understood. The roles of LH, hCG, and LHR are reasonably well delineated in the reproductive axis, although their contributions to cancer, particularly breast and ovarian, begs to be clarified. Such studies should utilize genomics, proteomics, and indices of proliferation, migration, and invasiveness in cell studies, and animal models and primary tumors as fully as possible. Lastly, further research is required on the importance and consequences of LHR expression in non-gonadal tissues. The future is indeed very bright for continued investigations.

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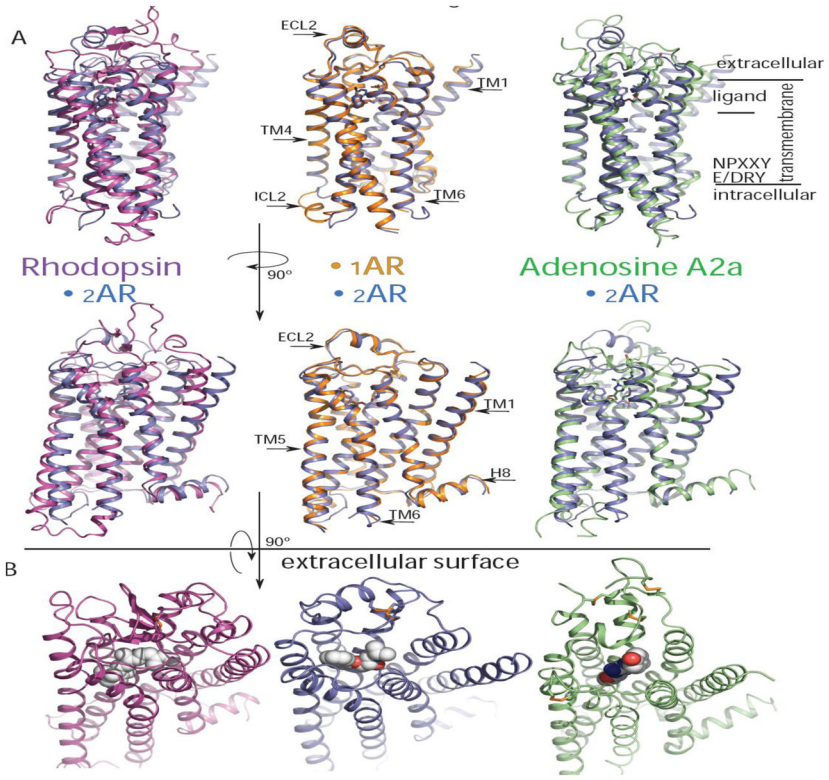

Fig. 1.

Crystal structures of four GPCRs in the inactive state. (A) Structures are shown for rhodopsin (purple), the β_1-adrenergic receptor (1AR, orange), the β_2-adrenergic receptor (2AR, blue), and the A_{2A} adenosine receptor (green) in two orientations. In the upper and lower rows, the structures of rhodopsin, the β_1-adrenergic receptor, and the A_{2A} adenosine receptor are each compared to that of the β_2-adrenergic receptor. The lower row shows the same structures depicted in the upper row after a rotation by 90° about the vertical axis. (B) Views of the same structures as in panel A following a 90° rotation about the horizontal axis (same coloring scheme as in A). The root mean square deviation of the TM regions is < 3A in these receptors. The structures emphasize both the highly conserved nature of major portions of these four GPCRs and the subtle differences that also exist. The figure was kindly provided by Dr. B.K. Kobilka and is reprinted with permission from Macmillan Publishers Ltd.: Nature (Rosenbaum et al., 2009).

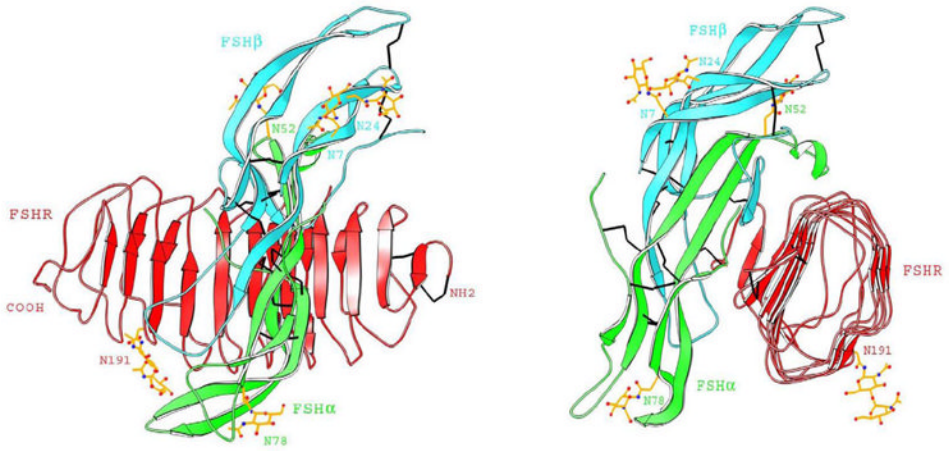

Fig. 2.

Crystal structure of the FSH-FSHR ECD complex. Two views of the complex, rotated by 90° about the vertical axis, are shown. The ECD is presented as red, and the FSH α and β chains are in green and cyan, respectively. The structure of the complex depicts the large contact surface area accompanying hormone-receptor binding and shows that both subunits contribute to receptor interaction. The figure was kindly provided by Dr. W.A. Hendrickson and is reproduced with permission from Macmillan Publishers Ltd.: Nature (Fan and Hendrickson, 1995).

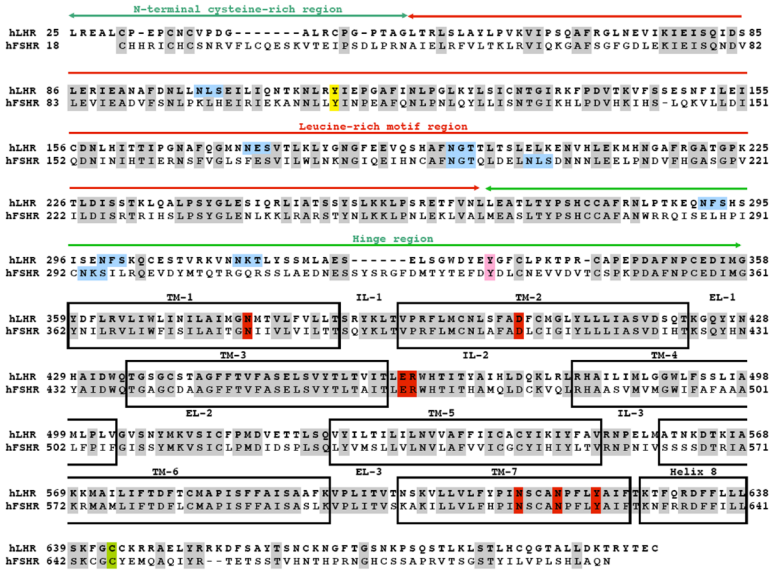

Fig. 3.

Amino acid sequences of human LHR and FSHR. Different portions of the receptor are indicated, including the N-terminal Cys-rich region, the LRR region, the hinge region with a Cys-rich component before TM1, the seven TM helices, the three ecls, the three icls, and helix 8 followed by the C-terminal tail. Gray shading: identical residues; blue boxes: consensus sequences for N-linked glycosylation; yellow: the Tyr that participates in dimerization for FSHR and is conserved in LHR; pink: potential site of sulfation; green: potential site of palmitoylation; and red: highly conserved residues of the rhodopsin/β_2-adrenergic family of GPCRs. The sequence was obtained from the web site, http://www.ensembl.org/index.html, and reproduced with permission from Elsevier (Ascoli and Puett, 2009).

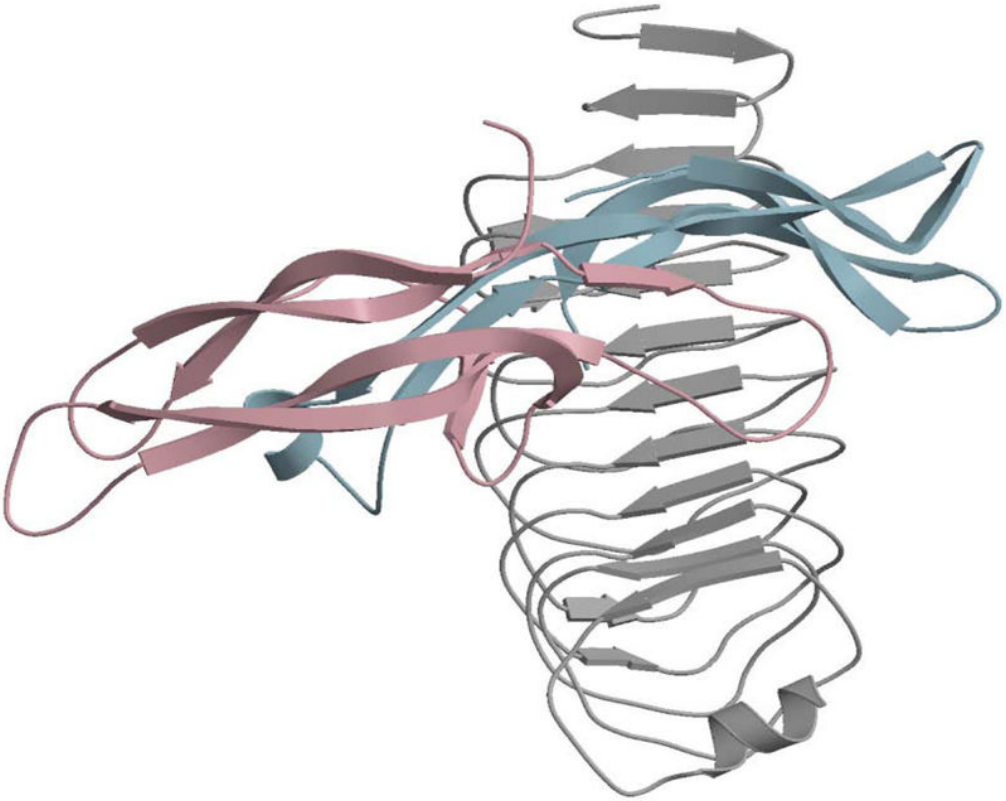

Fig. 4.

Model of hCG bound to the LHR ECD obtained by rigid body docking with the 3D DOCK program (Gabb et al., 1997). The model of the LHR ECD was based on comparative modeling using the MODELLER program (Sali and Blundell, 2003; Fiser and Sali, 2003) and the structures of the Nogo receptor (He et al. 2003), glycoprotein Ibα (Huizinga et al., 2002), and the FSHR ECD in complex with FSH (Fan and Hendrickson, 1995) and is thus devoid of the hinge region. The α and β subunits are shown in light blue and light pink, respectively, and it can be seen that both subunits, in particular loops 2 and 3 of α, loop 2 of β, and the β seatbelt loop, contact several LRRs of the ECD. The figure is reproduced with permission from Elsevier (Puett et al., 2007).

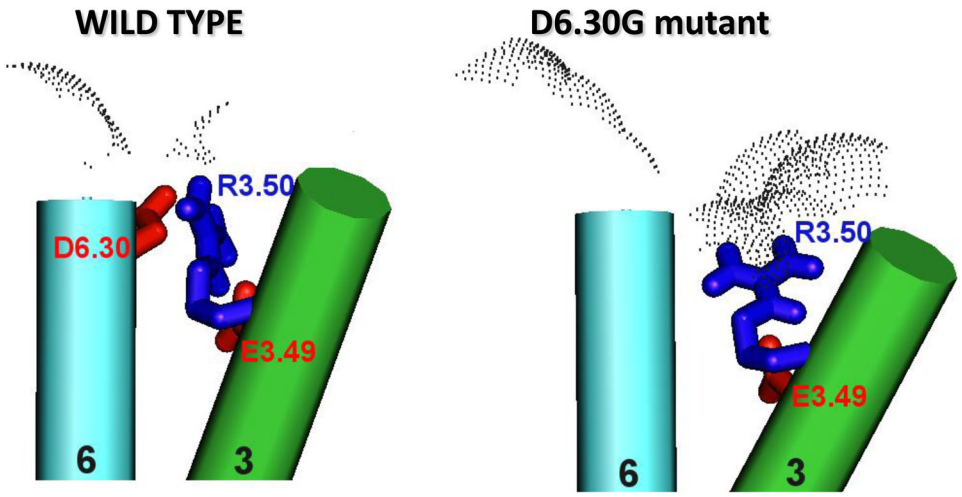

Fig. 5.

Cytosolic ends of TM3 and TM6 in wild type (left) LHR and the constitutively active LHR mutant, Asp-564-Gly (right). The interaction pattern of Arg-464 (3.50) is shown, and dots indicate that the SAS is 28 A^2 for wild type LHR and 120 A^2 for the Arg-464-Gly mutant.

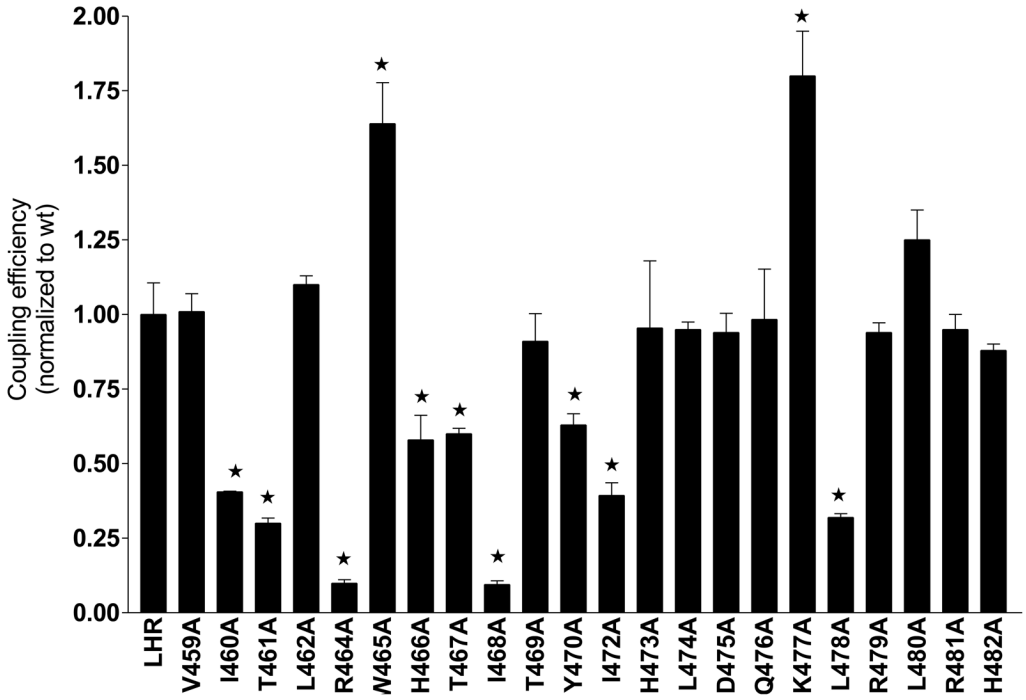

Fig. 6.

Coupling efficiencies of icl2 LHR mutants following Ala scanning mutagenesis. The coupling efficiency was determined from measurements of expression, binding, and signaling (cAMP production) in human embryonic kidney 293 cells. The coupling efficiency is defined as follows, $0.5[1 + (K_d/ED_{50})][R_{max}/B_{max}]$, where K_d is the hormone-receptor dissociation constant, ED_{50} represents the concentration of hCG required to achieve 50% maximal production of cAMP, R_{max} is the maximum amount of cAMP produced at a saturating concentration of hCG, and B_{max} is the receptor expression level; all values are normalized to that of wild type LHR. *, significantly different from wild type LHR ($P \leq 0.05$). Reproduced from Angelova et al. (2008) with the permission of The Endocrine Society, Copyright 2008.

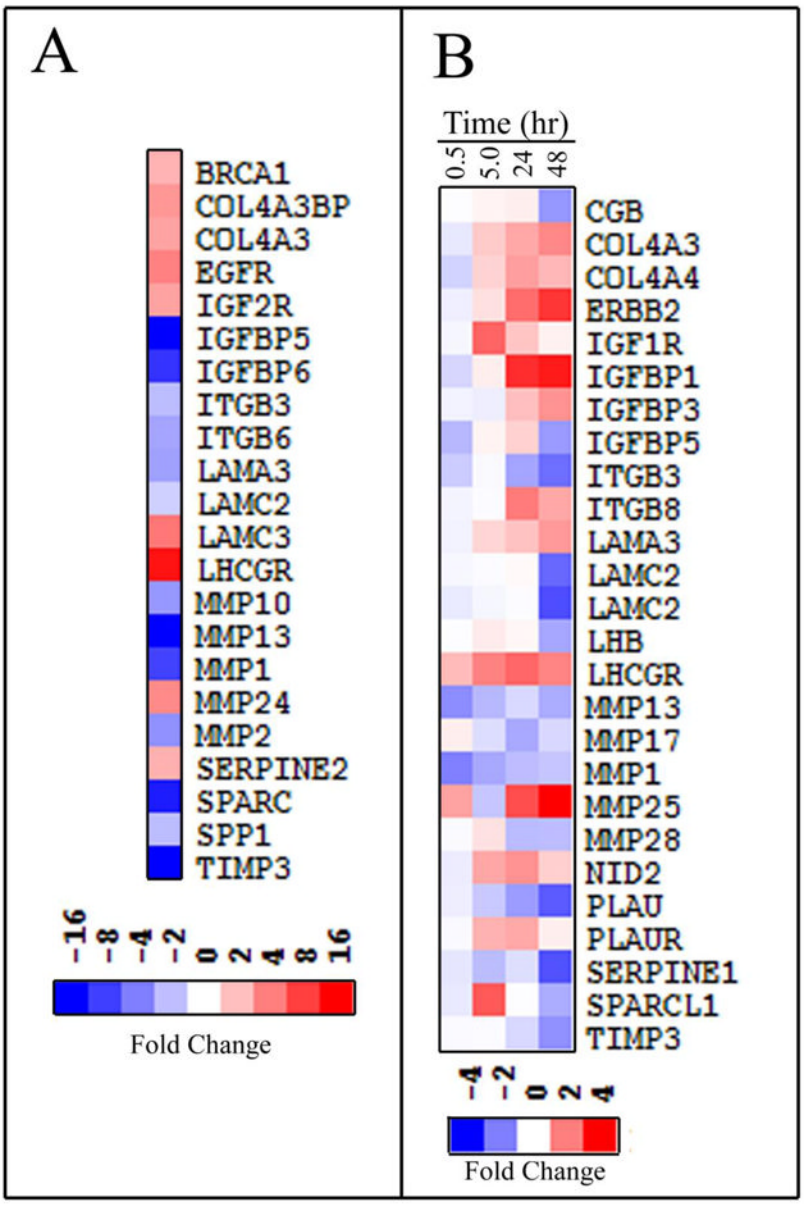

Fig. 7.

Gene expression in SKOV3 cells. RNA, prepared from mock transfected SKOV3 cells and stable transformants expressing about 12,000 receptors/cell, was converted to cDNA and gene expression determined by quantitative RT-PCR using ABI Taqman Expression Arrays. The data are presented using TreeView (http://rana.lbl.govEisenSoftware.htm). (A) Comparison of LHR+ cells to mock transfected cells. (B) Time-dependent changes in gene expression following incubation of LHR+ cells with LH (13 nM) for various times. The changes are relative to those of LHR+ cells at zero time. From Warrenfeltz et al. (2008) and reproduced with permission granted from the American Association of Cancer Research to authors publishing original figures in their sponsored journals.

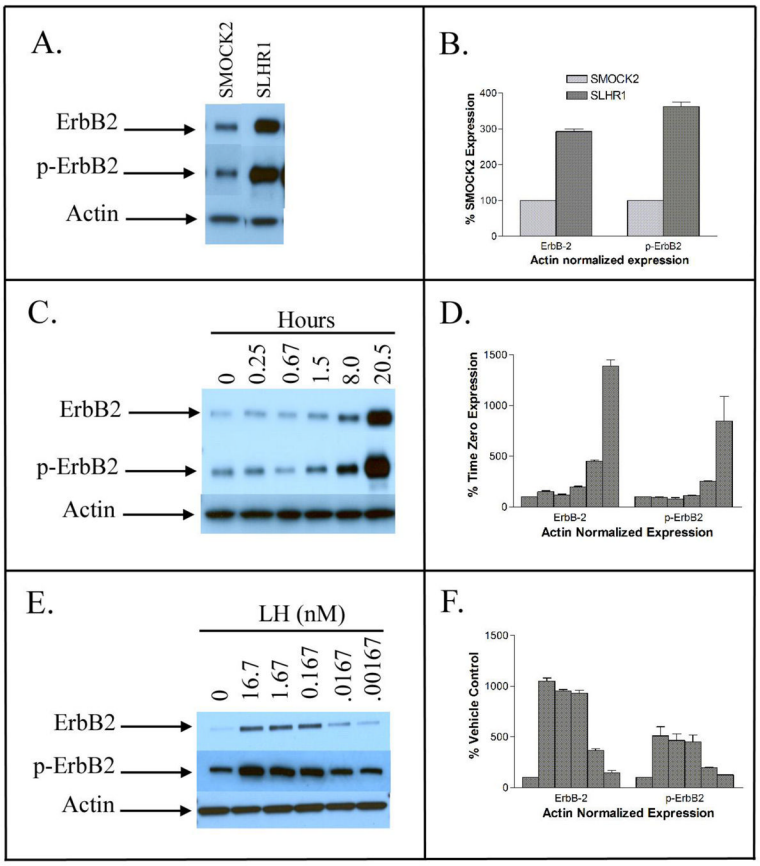

Fig. 8.

Protein expression (Western blots) in SKOV3 cells. Cellular proteins were separated on 4–20% polyacrylamide gels, then transferred to membranes, immunoblotted, and visualized using chemiluminescence. Actin was used as control in all experiments. (A) Western blots of LHR- cells (SMOCK2) and LHR+ cells (SLHR1) showing expression of ErbB-2 and phosphorylated ErbB-2 (p-ErbB2); (C) Western blots showing expression of ErbB-2 and p-ErbB-2 in LHR+ cells incubated with 16.7 nM LH for various time periods; and (E) Western blots showing expression of ErbB-2 and p-ErbB-2 after incubation for 18 h with different concentrations of LH. Panels (B), (D), and (F) show the results of densitometric measurements of ErbB-2 and p-ErbB-2 after normalization to actin; these panels correspond, respectively, to the blots presented in panels (A), (C), and (E). From Warrenfeltz et al. (2008) and reproduced with permission granted from the American Association of Cancer Research to authors publishing original figures in their sponsored journals.