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Structural Biology of Glycoprotein Hormones and their Receptors

Qing R. Fan[1] and Wayne A. Hendrickson[1,2]

[1]Department of Biochemistry and Molecular Biophysics and [2]Howard Hughes Medical Institute, Columbia University Medical Center, 630 West 168th Street, New York, NY 10032

Glycoprotein hormones regulate reproduction in vertebrates and exert their actions through specific G protein–coupled receptors on target cell surfaces. Structural information is now available for human chorionic gonadotropin (CG), follicle-stimulating hormone (FSH), and FSH bound to the extracellular binding domain of its receptor ($FSHR_{HB}$). The recently determined structure of a human FSH–$FSHR_{HB}$ complex provides an explanation for the specificity of glycoprotein hormones binding to their receptors, and it suggests hypotheses concerning the mechanism of transmembrane signal transduction.

Key Words: Glycoprotein hormones; G protein–coupled receptors; follicle stimulating hormone–receptor complex; crystal structure.

Introduction

The pituitary gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), are central to reproduction in vertebrates. In female mammals, FSH targets a receptor (FSHR) expressed only on granulosa cells to induce the maturation of ovarian follicles *(1–4)*. The levels of both FSH and FSHR surge at midcycle of the estrus as the luteinizing hormone (LH) triggers ovulation. In males, FSH stimulates Sertoli cell function and supports spermatogenesis *(1–4)*. FSH is used clinically to treat infertile patients. LH acts on the theca cells of the ovary and Leydig cells of the testis to regulate the concentration of gonadal steroid hormones *(3)*. LH is required for ovulation in the ovary *(3)*.

Production of FSH and LH is initiated in the hypothalamus, which synthesizes the decapeptide gonadotropin-releasing hormone (GnRH) (shown in Fig. 1 for FSH). GnRH targets the gonadotroph cells in anterior pituitary to secret both FSH and LH in a pulsatile fashion. The gonadotropins (FSH and LH) stimulate the production of sex steroid hormones estrogen, progesterone, and testosterone in the gonads. Circulating sex steroids prevent the synthesis of GnRH, thereby exerting a negative regulatory effect on the secretion of FSH and LH. In addition, the non-steroid hormones inhibin, activin, and folliltropin selectively inhibit and activate the secretion of FSH from the pituitary gland *(5,6)*.

FSH belongs to the glycoprotein hormone family, which also includes LH, chorionic gonadotropin (CG), and thyroid-stimulating hormone (TSH) *(7)*. Human CG (hCG) is a placental hormone required for the maintenance of early pregnancy *(7)*. TSH stimulates the synthesis and secretion of thyroid hormones in the thyroid gland *(8)*. The glycoprotein hormones are heterodimers composed of non-covalently associated α- and β-subunits. Within a given species, the α-subunit is common to all the hormones, but the β-subunit is unique and confers each hormone its functional specificity *(7)*.

The glycoprotein hormones elicit responses within target cells by binding to specific G protein–coupled receptors (GPCR) on the plasma membrane *(1,8,9)*. FSH and TSH bind to FSHR and TSHR, respectively; LH and its homolog CG bind to the same LHR. Upon ligand binding, the glycoprotein hormone receptor becomes activated and associates with a GDP-bound heterotrimeric G_s protein. The activated receptor serves as a nucleotide exchange factor, causing the exchange of GTP for GDP in Gα. This leads to the dissociation of Gβγ from GTP-bound Gα, which then proceeds to activate adenylyl cyclase for the production of cAMP. The cascade of signaling events eventually results in the synthesis of sex steroids.

The glycoprotein hormone receptors belong to a subfamily of GPCRs named leucine-rich-repeat-containing GPCRs (LGRs), which are characterized by a large extracellular domain with multiple leucine-rich repeats (LRRs) in addition to the rhodopsin-like seven-transmembrane (7TM) helices (Fig. 2) *(10)*. Unlike most other GPCRs, which respond to small-molecule ligands, the LGRs bind protein ligands including the glycoprotein hormones for type A receptors (FSHR, LHR, and TSHR), relaxin for type C receptors (LGR7 and 8) *(11,12)*, and bursicon for a *Drosophila* homolog of the type B LGRs (LGR4, 5, and 6) *(13)*. Alternatively, spliced variants of the glycoprotein hormone receptors have also been identified *(1,8,9)*. An unusual case is a

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Author to whom all correspondence and reprint requests should be addressed: Wayne A. Hendrickson, Department of Biochemistry and Molecular Biophysics, Columbia University, 630 West 168th Street, New York, NY 10032. E-mail: wayne@convex.hhmi.columbia.edu

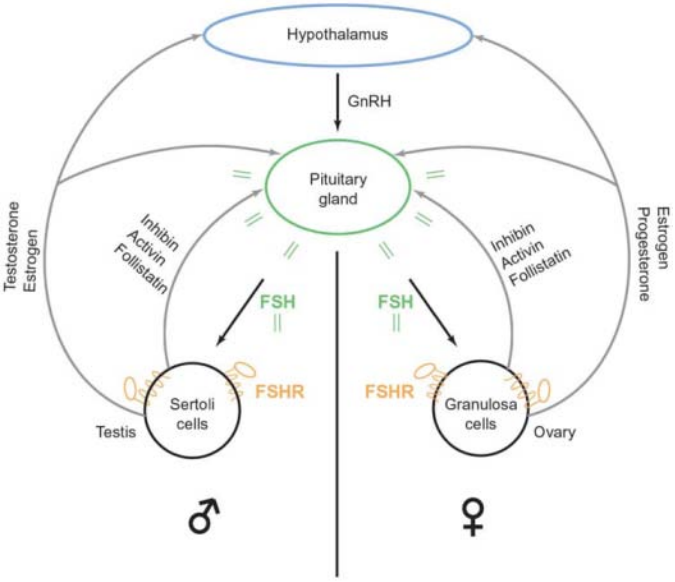

Fig. 1. Schematic diagram illustrating the feedback loop that regulates the secretion of FSH.

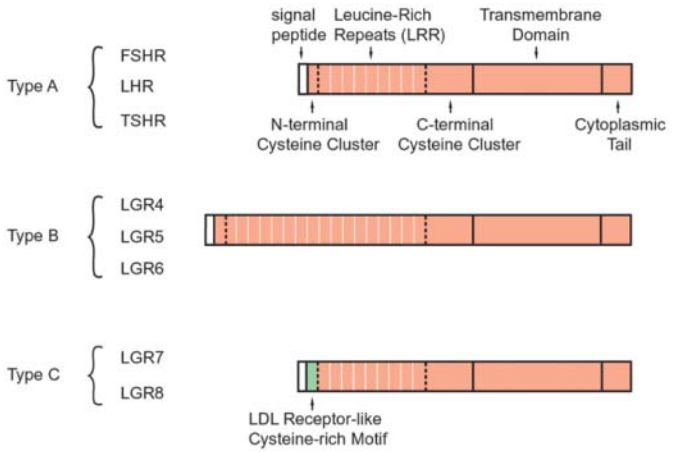

Fig. 2. Domain organizations of three subtyes of human LGRs.

FSHR variant with a single-transmembrane domain similar to growth factor receptors, which ex-hibits FSH binding activity in transfected cells *(14)*.

Glycoprotein hormones bind with high affinity and high specificity to the LRR-containing ectodomains of their cognate receptors *(1,8,9)*. The dissociation constants (K_d) of the hormone–receptor interactions are proportional to the circulation concentration of the hormones and are in the nanomolar range *(2,9)*. Receptor binding is largely separable from the receptor activation that leads to cAMP production. The transmembrane domains of these receptors are responsible for receptor activation and signal transduction. The recently determined crystal structure of human FSH bound to the hormone-binding domain of its receptor ($FSHR_{HB}$) *(14)* provides a molecular understanding of their interactions, which may be utilized to design FSH mimics as alternative agonists as well as contraceptive antagonists for both men and women. It also serves as a paradigm for the interactions of other glycoprotein hormones and receptors.

Structures of Glycoprotein Hormones

The crystal structures of two of the glycoprotein hormones have been determined to date, that of human CG *(15, 16)* and FSH *(17)*. Both reveal elongated molecules with similar folds for the α- and β-subunits (Figs. 3A,B). Each subunit has a cysteine-knot motif at its central core of extended hairpin loops. There are five disulfide bridges in the α-subunit and six in the β-subunits. Extensive subunit interfaces involve two interchain β-sheets and bury 3860 $Å^2$ and 4080 $Å^2$ of solvent-accessible surface area in CG *(15,16)* and FSH *(17)*, respectively. The heterodimeric association is further stabilized by a disulfide-tethered segment from the β-subunit, which embraces the α-subunit in a seatbelt-like fashion. The structure of FSH is similar to that of CG, and differs only in the conformations of the termini and the β*L2* loop *(17)*.

The glycoprotein hormones have an abundance of carbohydrates. Glycosylation has been implicated in the stability, circulatory lifetime, and potency of these hormones *(18–20)*. Each subunit has one (LHβ and TSHβ) or two (common α, FSHβ, and CGβ) N-linked glycosylation sites. There are also four O-linked glycosylation sites on the carboxyterminal of CG β-subunit, a segment missing in other glycoprotein hormones. The structure of CG alone was determined using recombinant protein expressed in mammalian cells and partially deglycosylated by HF treatment *(15,16)*. A structure is also available for fully glycosylated CG complexed with two Fv fragments specific for the α- and β-subunits *(21)*. Neither the carbohydrates nor the Fv molecules appreciably affect the structure of CG. The structure of free FSH was obtained from insect cell–expressed protein, with a T26A mutation introduced in the β-subunit to eliminate glycosylation at the βN24 site *(17)*. Comparison of the structures of CG and FSH indicates that glycosylation is not responsible for their conformational differences *(17)*. In both CG and FSH structures, contacts were observed between the sugar group at αN52 and neighboring β-subunit residues, suggesting that the carbohydrates at this site may contribute to the stability of the heterodimer *(15–17)*.

Structure of the Human FSH–$FSHR_{HB}$ Complex

The crystal structure of human FSH complexed with the hormone-binding domain of its receptor has recently been determined (Fig. 3C) *(15)*. The recombinant FSH–$FSHR_{HB}$ complex was produced through secretion from insect cells that had been infected with a baculovirus vector constructed to encode both an FSHβ–FSHα fusion protein and a hormone-binding portion of the FSHR ectodomain. The α- and β-subunits of the heterodimeric FSH were covalently stabilized with a Gly/Ser-rich linker. Similar single-chain FSH and CG constructs, but with different linkers, have previously been shown to retain receptor-binding activities *(23–25)*. The extracellular domains of all glycoprotein hormone receptors consist of a series of LRRs flanked at each end by

cysteine clusters (Fig. 2). The hormone-binding domain of FSHR ($FSHR_{HB}$) contains the N-terminal cysteine cluster and the LRRs (aa 1–268). The C-terminal cysteine-rich linker region is not required for hormone binding. Partial deglycosylation removed approx 20% of the sugar on the FSH–$FSHR_{HB}$ complex.

The $FSHR_{HB}$ structure is a slightly curved solenoid formed by 10 LRRs, which are highly irregular in length and conformation. As predicted from sequence considerations *(26)* and as found in other LRR structures *(27)*, conserved leucine, isoleucine, and phenylalanine residues form its hydrophobic core. The overall fold of $FSHR_{HB}$ is likely to be conserved in LHR and TSHR because the leucines and other hydrophobic residues are conserved at equivalent positions in the sequences of LHR and TSHR, as are all the repeat irregularities.

The $FSHR_{HB}$ structure displays two parts with distinctively different curvatures. The C-terminal portion (repeats 7–10) has the signature horseshoe-like curvature of LRR proteins whereas the N-terminal portion (repeats 1–7) is nearly flat. The N-terminal flanking sequence (residues 18–46) is an integral part of the LRR architecture; it contains the first of the LRR repeats, an additional antiparallel β-strand and two disulfide bridges (Cys18–Cys25 and Cys23–Cys32).

FSH binds in a hand-clasp fashion to the curved inner surface of $FSHR_{HB}$, making contacts with all 10 β-strands of the repeat structure (Figs. 3C and 4A). The receptor wraps around the middle section of the hormone molecule and interacts with the C-terminal segments of both FSHα- and β-subunits, as well as the α*L*2 and β*L*2 loops (Figs. 3C and 4A). The hormone is oriented such that loops β*L*1 and β*L*3 extend out from the C-terminal tips of the receptor inner sheet, whereas loops α*L*1 and β*L*3 protrude away from the base (Fig. 3C).

Both FSHα- and β-subunits are involved in the interaction with $FSHR_{HB}$ (Fig. 4A). The resulting interface is large (2600 $Å^2$) and has a high charge density (1.1 charges per nm^2) (Fig. 4B). The key elements of the interactions between FSH and $FSHR_{HB}$ involve the packing of two rather flat surfaces, one formed by the N-terminal seven β-strands of $FSHR_{HB}$, and the other includes the C-terminal segments of FSHα and FSHβ. The tip of the β*L*2 loop contacts the curved C-terminal repeats of $FSHR_{HB}$. The α*L*2 loop with its helical segment clamps over the top of the $FSHR_{HB}$ structure and contacts loops adjacent to the β-sheet.

Carbohydrates do not participate in receptor–hormone interactions. The structure of the FSH–$FSHR_{HB}$ complex reveals N-linked carbohydrates at all four potential glycosylation sites on the hormone (FSHα N52 and N78; FSHβ N7 and N24), and at the one confirmed site on the receptor (N191), which is conserved among all glycoprotein hormone receptors across different species *(28)*. All are distant from the interface. Natural mutations located at or near the conserved glycosylation site of FSHR (N191I, A189V) have been shown to cause inactivation of the receptor *(3)*, possibly by affecting the proper folding of FSHR *(28)*. As in the free FSH structure, the carbohydrate group at N52 of FSHα contacts the FSHβ chain in the FSH–$FSHR_{HB}$ complex, again suggesting its role in the thermostability of the heterodimer. Consistent with this observation, and contrary to previous implication that glycosylation at N52 of hormone α-chain is involved in signaling, mutational deglycosylation of this site in disulfide-stabilized hCG has no effect on activity *(29)*.

Universality and Specificity in the Hormone–Receptor Interaction

The interactions between FSH and $FSHR_{HB}$ suggest that the binding mode is universal among all glycoprotein hormone and receptors. First, both the structures of the hormone and the hormone-binding domain of the receptor are expected to be essentially the same among their family members because of high sequence homology. The common ligand structure has been demonstrated by the similar structures of human FSH *(18)* and CG *(16,17)* and the presence of an identical α-chain within a species provide additional evidence for a common ligand structure. Second, the structural elements found at the receptor–hormone interface of FSH–$FSHR_{HB}$ complex have also been implicated in the interaction of other receptor–hormone pairs. Finally, many residues of $FSHR_{HB}$ involved in contacting the common α-subunit of hormone are conserved among receptor homologues, consistent with a universal binding mode.

Residues with complementary charged groups contribute to the universal interactions at the hormone–receptor interface. These include salt bridges between invariant acidic residues from the receptor (D150 and D153 in FSHR) and basic residues from the common α-subunit (K91 and K51) (Fig. 4B, black dotted lines; Fig. 5), as well as charge-mediated interactions involving FSHR residue D81 (conserved in LHR) and residues R42 and R45 from the α*L*2 loop of FSH (Fig. 4B, yellow dotted lines; Fig. 5). These findings are consistent with loss-of-function studies by charge reversal mutagenesis of ionizable amino acids E132 and D135 in LHR *(30)* (residues corresponding to D150 and D153 in FSHR), alanine mutations of FSHα residues K51 *(31)* and K91 *(32)*, as well as peptide-inhibition studies of the α*L*2 regions of both human CG and TSH in receptor binding *(33,34)*.

Charged interactions also mediate the contacts between conserved residues in the hormone β-chain and the receptor. An example is the salt bridge formed between K104 of $FSHR_{HB}$ and D93 of FSHβ (Fig. 4B, yellow dotted lines; Figs. 5 and 7). Mutational studies indicate that the invariant Asp residue in the β-chain of both FSH (D93) and CG (D99) is important for receptor binding *(34,35)*. The observed interaction also explains why substituting K104 of FSHR with Asn of LHR increases the sensitivity of mutant FSHR toward hCG without affecting its binding to FSH *(36,37)*.

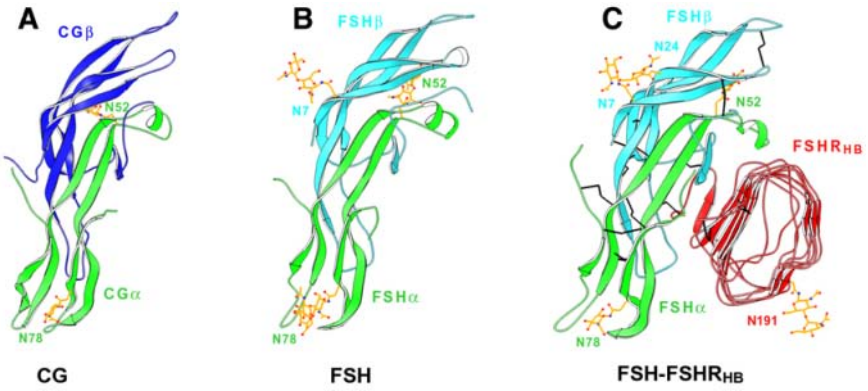

Fig. 3. Ribbon diagrams showing the crystal structures of (**A**) free human CG, (**B**) free human FSH, and (**C**) human FSH bound to $FSHR_{HB}$. Carbohydrates are in yellow. Disulfide bonds in the FSH–$FSHR_{HB}$ structure are in black.

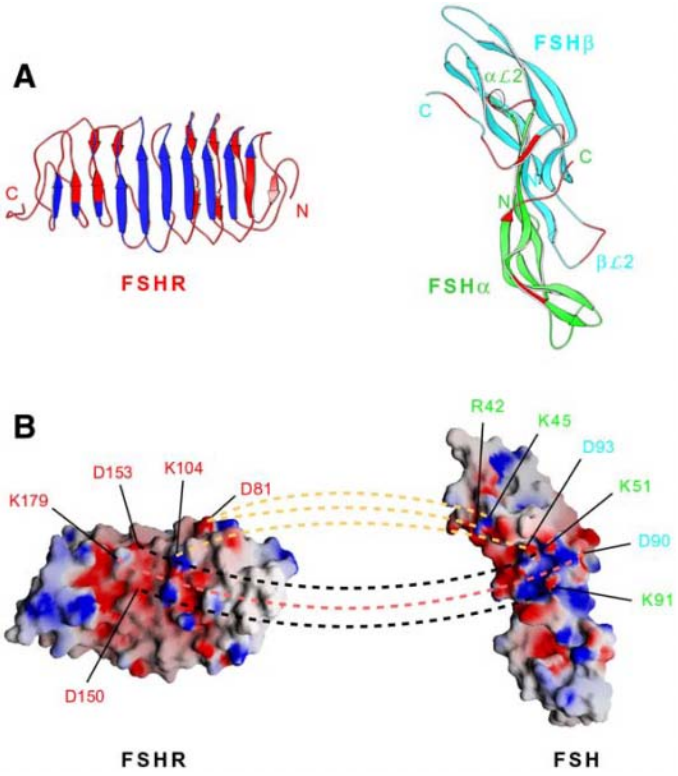

Fig. 4. Recognition of FSH by $FSHR_{HB}$. (**A**) Ribbon diagrams of $FSHR_{HB}$ (left; red) and FSH (right; α in green; β in cyan). Blue regions in $FSHR_{HB}$ are involved in hormone binding. Red regions in FSH are involved in receptor binding. (**B**) Electrostatic potential surface of $FSHR_{HB}$ (left) and FSH (right). Dotted lines mark the key interactions between charged residues of the receptor and hormone.

An Asn residue at position 104 of FSHR could still form a hydrogen bond with D93 of FSHβ; therefore, it would not negatively affect the interaction between mutant FSHR and FSH.

Extensive interactions between the C-terminal regions of FSHα (aa 88–92) and and the bound receptor also support a common binding mode between glycoprotein hormones and receptors. Many of the contacts involve residues that are conserved among glycoprotein hormone receptors. One of the key features of the FSH–$FSHR_{HB}$ interface is an aromatic ring-stacking interaction between Y88 of the hormone α-chain and Y124 of the receptor. An aromatic residue is present in both human LHR (Y127) and TSHR (F130) (Figs. 5 and 7). A total of 106 $Å^2$ of solvent accessible surface area from Y88 of FSHα is buried upon receptor binding. The importance of Y88 in receptor interaction is demonstrated by deletion and alanine mutagenesis experiments of the C-terminal amino acids of hCGα (aa 88–92) *(32,39)*.

The interactions between the glycoprotein hormones and their corresponding receptors are highly selective, with very few cases of cross activity. Because glycoprotein hormones within a certain species all have the same α chain, they are distinguished by their β-chains. The C-terminal seatbelt segments of β chain have been implicated in specificity determination *(40–45)*. The structure of FSH–$FSHR_{HB}$ complex demonstrates that receptor specificity is jointly mediated by hormone α- and β-subunits.

Sequence comparison has been used to identify potential determinants for specificity among the interactions at receptor–hormone interface. Residues that are involved in direct contacts and are substantially buried upon binding (<40% solvent exposed) are the candidates being considered (Figs. 6A,B,C, shaded). Sequence variability in the human glycoprotein receptors (FSHR, LHR, and TSHR) indicates that six such residues in $FSHR_{HB}$ (L55, R101, N106, Q152, K179, and I222) have different amino acids in all three receptors (Figs. 6A,C, dark gray with stars). Eight $FSHR_{HB}$ residues have one difference, and three are completely conserved (Figs. 6A,C, light gray). Sequence variation in their binding partners in the hormone indicates that most of these receptor residues contact the common α-chain and/or conserved β-chain residues (Fig. 6B, light gray; Fig. 6D). Four receptor residues (L55, E76, R101, and K179) contact FSHβ residues, which are all different among the hormone β-chains

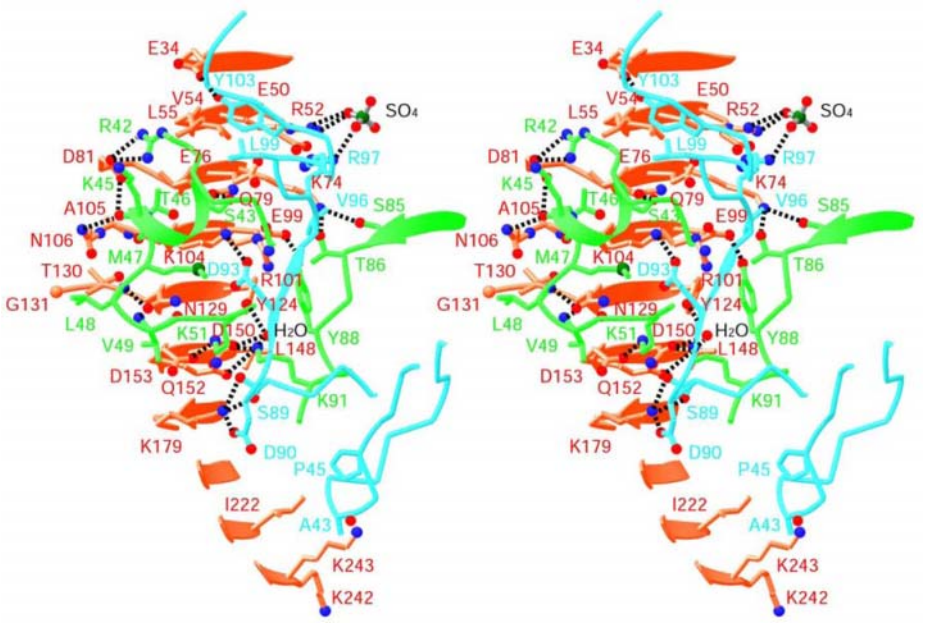

Fig. 5. Stereo view of the direct contacts (distances < 4 Å) observed at the receptor–hormone interface of FSH–$FSHR_{HB}$ complex.

(Fig. 6B, dark gray with stars; Fig. 6D), and one (I222) interacts with a FSHβ residue that shows one difference (Fig. 6B, light gray with stars; Fig. 6D). In summary, five $FSHR_{HB}$ residues (L55, E76, R101, K179, and I222) both vary among human receptors and interact with FSH residues that vary among the counterpart hormones. These receptor residues constitute four predominant sites that contribute to specificity (L55, K179, I222 and the combination of E76 and R101).

The interactions between receptor residues 55 and 179 and the hormone seatbelt region determine the selection between FSH and TSH vs LH/CG. A common feature of the specificity pockets for L55 and K179 is the involvement of both discriminating β-chain seatbelt residues and common α-chain residues. The molecular surface of FSH shows a shallow pocket for the side chain of L55, which forms hydrophobic interactions with L99 and Y103 of FSHβ and with the aliphatic part of R42 from FSHα (Fig. 7). Substituting L55 of FSHR with a Tyr residue found in LHR resulted in drastic loss of function and hormone binding for the receptor *(37)*. The structure of the FSH–$FSHR_{HB}$ complex shows that a large Tyr residue at position 55 could not be accommodated in the shallow pocket for L55 because of steric hindrance. The Tyr residue of LHR would be compatible with the smaller and polar residues found at the same location in LHβ or CGβ (D105 and T109). Indeed, exchange of the intercysteine loop of FSHβ (^{95}TVRGLG^{100}) with that of LHβ (^{101}GGPKDH^{106}) impairs receptor binding by FSH *(44)*.

The structure of the FSH–$FSHR_{HB}$ complex also provides the molecular basis for extensive mutagenesis studies showing that K179 of FSHR functions as a negative determinant to prevent the binding of CG/LH to FSHR *(37,38)*. A channel on the surface of FSH hosts the side chain of K179 (Fig. 7). Residues K51 and K91 of FSHα line the sides of the channel; residues S89 and D90 of FSHβ cap the top and form three hydrogen bonds with K179 of FSHR (Fig. 7). An Arg residue at the corresponding position of S89 in CGβ/LHβ would cause steric interaction and charge–charge repulsion with K179 of FSHR. These interactions will inhibit the interaction between CG/LH and FSHR. The structural observations are in agreement with studies where mutating K179 to a Gly found in LHR gave the mutant FSHR a gain of sensitivity toward hCG *(37)*, and replacing FSHβ residues ^{88}DSDS^{91} with their LHβ counterparts (^{94}RRST^{97}) allowed FSH to acquire LHR binding activity *(44)*.

A third specificity-determinating site involves the structurally adjacent E76 and R101, which interact with R97 and V96 from FSHβ, respectively (Figs. 5 and 7). Charged side chains do not make hydrogen bonds here, but repulsion expected from FSHR E76 and R101 interacting with the TSHβ Glu and His counterparts of R97 and V96 may explain FSH versus TSH selectivity.

Finally, there are potentially discriminating interactions between α-strand 9 and hormone loop β*L2*, the Keutmann loop implicated in hCG interactions with its receptor *(46)*. Although the interactions between I222 and the β*L2* loop of FSH do not appear to be strongly discriminating, the neighboring residue K243 in $FSHR_{HB}$ structure makes polar interactions with the main chain of FSHβ residue A43 at the peripheral of the hormone–receptor interface (Figs. 5 and 7). The presence of a positively charged K44 in the lengthened β*L2* loop of TSH would cause charge repulsion with K243 of FSHR, but is complementary to E251 found in TSHR. The interaction between β*L2* loop and the receptor may thus participate in FSH versus TSH specificity.

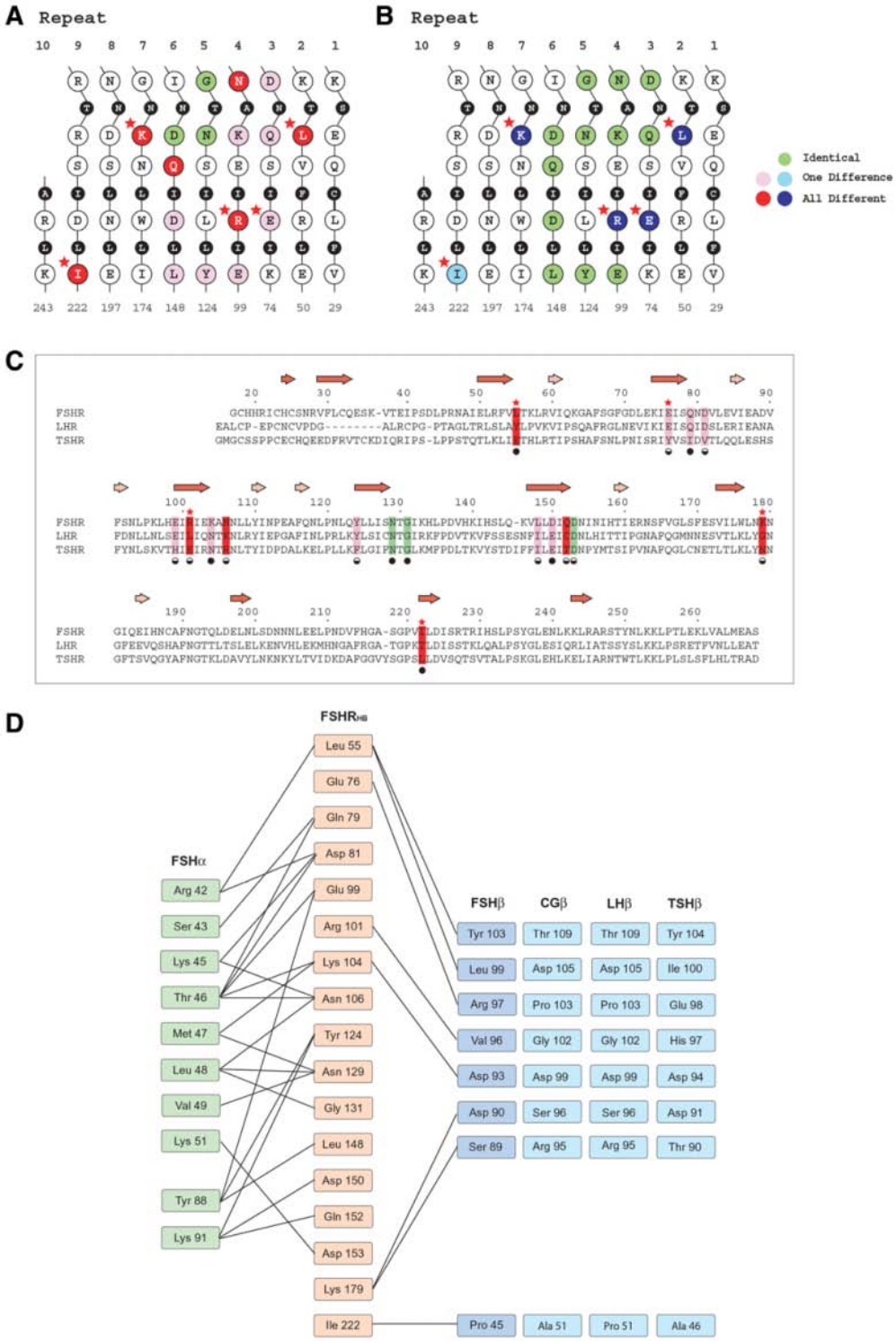

Fig. 6. Specificity of glycoprotein hormone and receptor recognition as analyzed by sequence variation. In (**A**) and (**B**), the concave face of the $FSHR_{HB}$ structure is shown as an unrolled sheet. Residues are represented by filled circles for side chains that point toward the hydrophobic core and by open circles for those that are solvent-exposed. Residues of $FSHR_{HB}$ that are involved in direct contact with FSH and become less than 40% solvent-exposed upon hormone binding are shaded according to (**A**) sequence variation among the three human glycoprotein hormone receptors (FSHR, LHR, and TSHR) or (**B**) sequence variation at their interaction partners in the hormone (FSHβ, CGβ/LHβ, and TSHβ). Contacts with the common α-chain are regarded as conserved interactions. (**C**) Alignment FSHR, LHR, and TSHR sequences in the region of the hormone-binding domain. Arrows above the sequences represent β-strands located at the concave (dark gray) or convex (light gray) face of $FSHR_{HB}$ structure. Residues are highlighted according to the same coloring scheme as in **A**. The fractional solvent accessibility is indicated by a filled circle if it is less than 10%, and half-filled circle if it is 10–40%. (**D**) Direct contacts (black lines) between residues of $FSHR_{HB}$ and FSHα/FSHβ. Only residues of $FSHR_{HB}$ that are highlighted in **A** and **C** are shown. The corresponding amino acids in human CGβ, LHβ, and TSHβ are listed adjacent to FSHβ residues.

Conformational Adaptability of FSH in its Receptor Complex

Upon receptor binding, FSH undergoes a concerted conformational change that affects the FSHα C-terminus and protruding loops at one end of the elongated molecule (Fig. 8A). The hormone conformation also becomes significantly rigidified in its receptor complex. The most significant change occurs at the C-terminal region of FSHα (aa 88–92), which is more than 20 Å away from the free state (Fig. 8B). In the structure of free FSH, this region is highly flexible, and adopts different conformations in the two copies found in the crystal (Fig. 8B). Furthermore, the last two residues of FSHα are disordered, as are the last three α-chain residues in free CG. In contrast, the C-terminus of FSHα has nearly identical conformation in the two copies of FSH–$FSHR_{HB}$ complex, and is completely ordered (Fig. 8B). This segment is buried at the receptor–hormone interface and makes extensive contacts with the receptor (Fig. 7). Receptor binding thus locks the FSHα C-terminus into an orientation favorable for receptor interaction.

The basal loops of FSH (α*L*1, α*L*3, and β*L*2) are also fixed in their receptor-bound state. The contacts between β*L*2 and three C-terminal LRRs of $FSHR_{HB}$ dictate the conformation of β*L*2. The interactions between β*L*2 and the neighboring α*L*3 as well as those between α*L*3 and α*L*1 then force the receptor-induced deformation of β*L*2 onto α*L*3 and α*L*1. The conformation assumed by these basal loops upon receptor binding is presumably one favorable for receptor activation.

Dimerization of FSH–$FSHR_{HB}$ Complexes

The FSH–$FSHR_{HB}$ complex forms dimers in the crystal and at high concentrations in solution. The two independent FSH–$FSHR_{HB}$ complexes in the crystal are related by a pseudo two-fold rotation axis, which is approximately perpendicular to the receptor tube axis (Fig. 9). The dimer interface involves the three-stranded β-sheet (LRRs 2–4) on the outer surface of the $FSHR_{HB}$ structure and the interactions are predominantly hydrophobic. The completely conserved Y110 residue mediates a majority of the carbon–carbon contacts, suggesting that other glycoprotein hormone receptors may form dimers in a similar way. The solvent accessible surface area buried at the dimeric interface is relatively small (940 $Å^2$ total), which is consistent with a weak association detected in solution ($K_d \approx 400$ μ*M*) by chemical crosslinking, analytical ultracentrifugation and light scattering.

Implications of the Structure for Receptor Activation

Several models have been proposed for the activation mechanism of glycoprotein hormone receptors. One involves hormone-induced conformational changes in the extracellular domain that are transmitted to the 7TM domain for activation *(47)*. A second proposes that hormone binding switches the ectodomain from a tethered inverse agonist to a full agonist of the transmembrane region of the receptor *(48,49)*. A third hypothesizes that the hormone bound to the ectodomain contacts an additional binding site in the 7TM domain for activation *(26,47,50)*.

The model of the ectodomain having an inhibitory effect on the 7TM domain is based on the observations that constitutively activating mutations exist in the ectodomain of the receptor *(48,49)*. These activating mutations are located in the cysteine-rich linker region between the LRRs and the 7TM domain. It is proposed that the activating mutations and the hormone achieve the same goal by turning the ectodomain into an activated agonist of the 7TM domain *(48,49)*. Although this model does not require direct contact between the hormone and 7TM domain for activation, it does not preclude such an interaction.

The crystal structure of FSH–$FSHR_{HB}$ complex provokes the suggestion that the α-tips of the receptor-bound hormone may directly contact the 7TM domain and the linker segment (Fig. 10). The linker region is also involved in receptor activation as indicated by the constitutively activating mutations and the requirement of tyrosine sulfation at a site close to the membrane *(51)*. Direct hormone contact with the 7TM domain is supported by experiments such as chimeric exchanges *(47)*, antibody occlusion *(50)*, EL2-peptide inhibition of signaling *(50)* and α-tip antibody access, and signal enhancing β*L*1 mutations *(52)*.

The observation of a dimer of the FSH–$FSHR_{HB}$ complex in the crystal and the detection of weak dimers in solution suggests that dimerization may have functional consequences for glycoprotein hormone receptors. The weak dimeric interaction between the soluble FSH–$FSHR_{HB}$ complex (K_d of 400 μ*M*) is expected to be enhanced when the receptors are tethered to the membrane *(53)*. Indeed, self-association of LHR has been directly measured by co-immunoprecipitation of differentially tagged receptors *(54)* and by fluorescence-resonance energy transfer experiments *(55)*. An alternatively spliced variant of human LHR has also been shown to regulate the cell surface expression of wild-type LHR by selectively forming dimers with immature receptors *(56)*.

The recent discovery of both homo- and heterodimers of other GPCRs also suggests that dimerization may play a role in GPCR signal transduction in general *(57)*. A reconstitution experiment has demonstrated that the activated leukotriene B_4 receptor forms a pentameric assembly consisting of one heterotrimeric G protein and one dimeric receptor *(58)*. In the case of the metabotropic $GABA_B$ receptor, a heterodimeric assembly of the $GABA_BR1$ and $GABA_BR2$ subunits is required for receptor function, and dimerization is facilitated by coiled-coil domains in the cytoplasmic region *(59)*. The crystal structure of the metabotropic glutamate receptor (mGluR) ectodomain reveals a ligand-binding pocket located away from the transmembrane domain; it also shows multiple conformations for the homodimeric extracellular domain with the activated form being selectively stabilized by glutamate binding *(60)*.

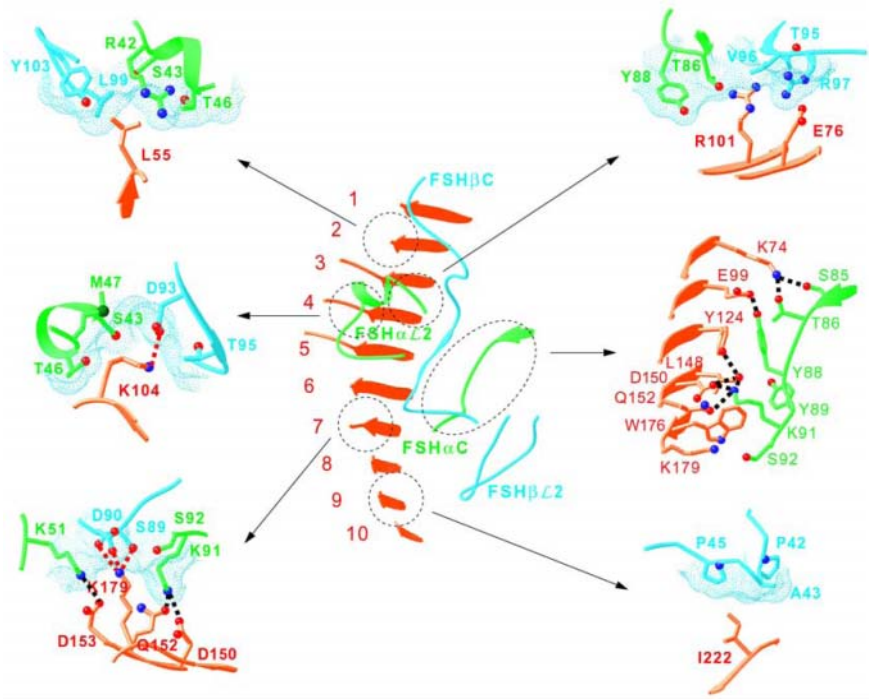

Fig. 7. Ribbon diagram in the center shows a top view of the regions of $FSHR_{HB}$ (red), FSHα (green), and FSHβ (cyan) that are involved in direct contacts at the receptor–hormone interface. Panels surrounding the central figure provide detailed views of the interactions highlighted by the circles. These include specificity pockets for L55 and K179 of $FSHR_{HB}$, specific interactions involving E76, R101, and I222 of $FSHR_{HB}$, and universal contacts observed at K104 of $FSHR_{HB}$ and the C-terminal tail of FSHα.

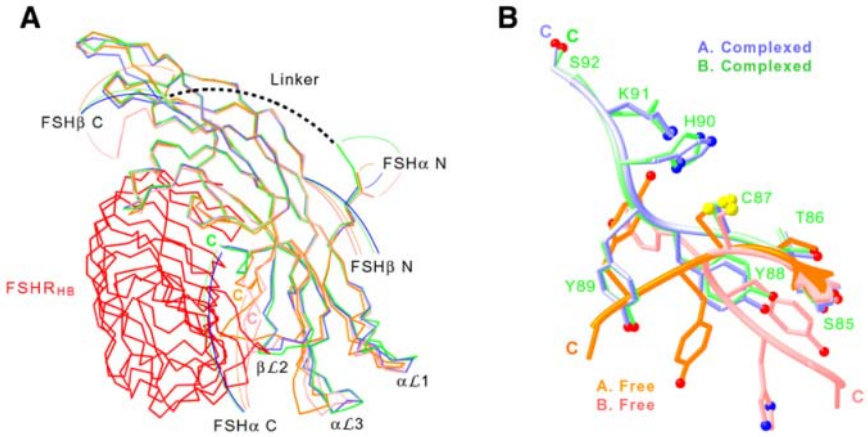

Fig. 8. Receptor-induced conformational changes in FSH. (**A**) Cα trace superposition of four independent copies of FSH. The FSH molecules in green and blue are receptor-bound, with the one in green complexed to the $FSHR_{HB}$ molecule shown in red. The two protomers of free FSH are in orange and pink. (**B**) Detailed view of the different conformations at the C-terminal region of α-chain in free (orange and pink) and receptor-bound (blue and green) FSH.

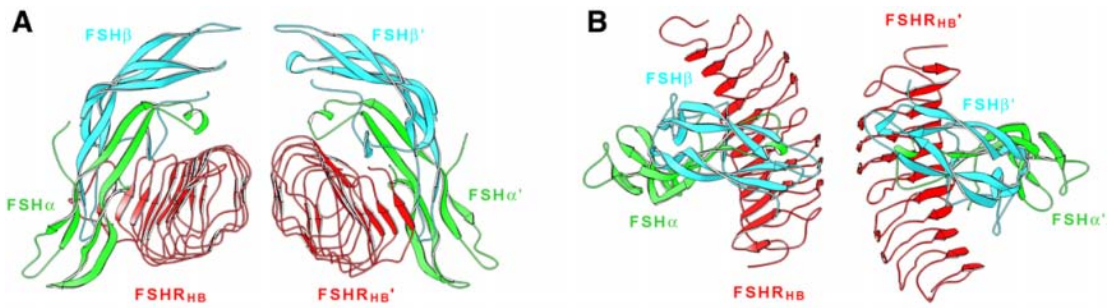

Fig. 9. Dimer of the FSH–$FSHR_{HB}$ complex in (**A**) side view and (**B**) top view.

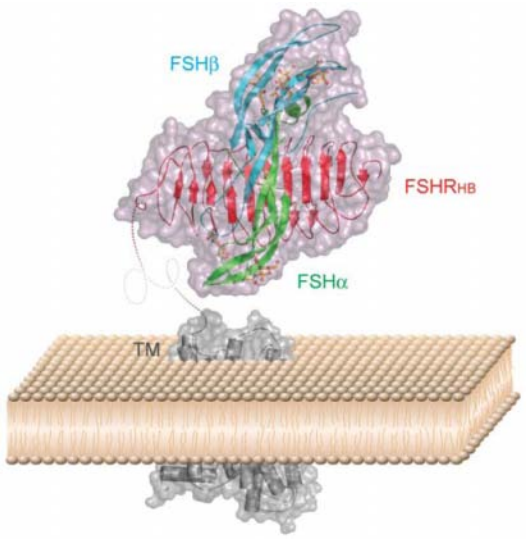

Fig. 10. Ribbon and surface representation for the activation model of glycoprotein hormone receptors. The FSH–$FSHR_{HB}$ complex structure is positioned such that the FSHα tip contacts the transmembrane (TM) domain. The TM domain is modeled based on bovine rhodopsin structure.

Dimers like the one formed by the FSH–$FSHR_{HB}$ complex in crystal may also be involved in glycoprotein hormone receptor signaling. If both the receptor-bound hormones in the dimer are contacting the 7TM domain, the receptor C-termini in the dimeric complex may be too far apart (approx 70 Å) to accommodate interactions between the 7TM domains (Fig. 11B). We propose that activation of one of the complexes in the dimer is sufficient for signaling (Fig. 11A). An alternative scenario where the hormone in a dimeric complex contacts the 7TM domain of the neighboring receptor for activation (Fig. 11C) is consistent with cellular assays where co-expression of a mutant receptor defective in hormone binding and another defective in signal transduction reconstitutes ligand-induced cAMP production *(61,62)*.

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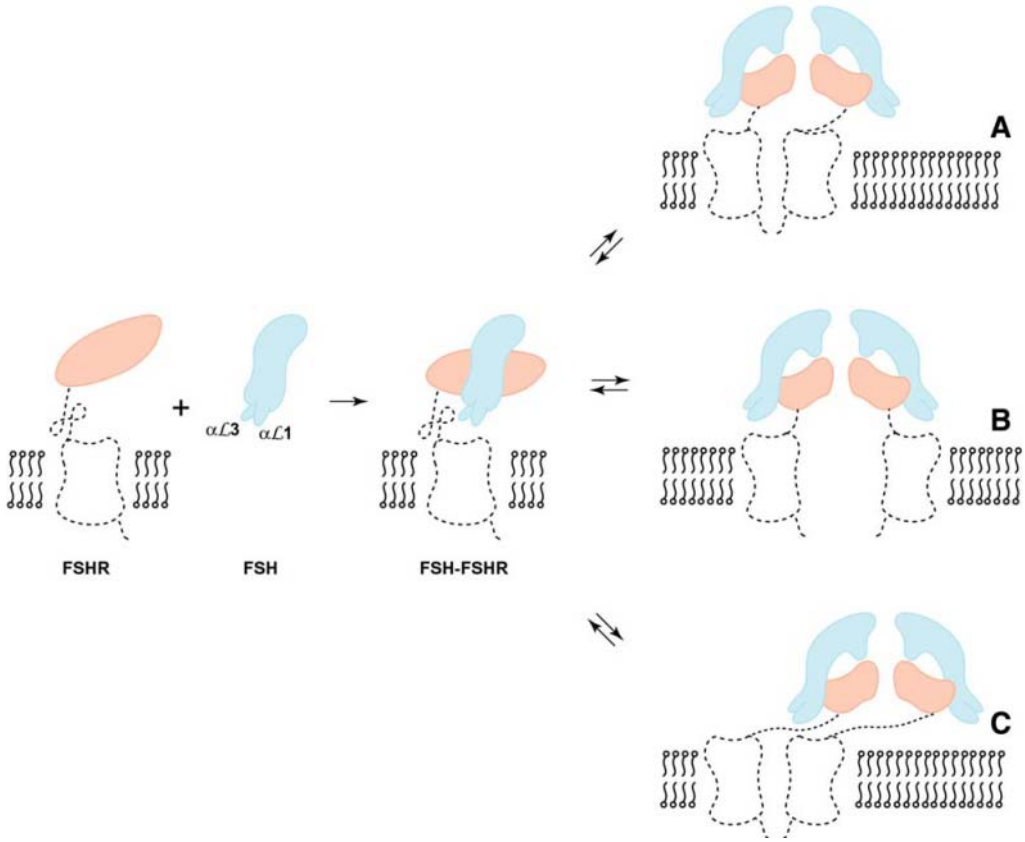

Fig. 11. Schematic diagram illustrating three potential scenarios (**A**, **B**, **C**) in which dimerization is involved in glycoprotein hormone receptor signaling. Projections of the FSH–$FSHR_{HB}$ complex and bovine rhodopsin structures are used to represent the hormone (light gray), the hormone-binding domain (dark gray), and the linker and transmembrane domain (black dotted trace) of the receptor.

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