Published in final edited form as:

Mol Cell Endocrinol. 2007 January 2; 260-262: 153–162.

Role of the intracellular domains of the human FSH receptor in G_{as} protein coupling and receptor expression

Alfredo Ulloa-Aguirre^{a,*}, Aída Uribe^a, Teresa Zariñán^a, Ismael Bustos-Jaimes^b, Marco A. Pérez-Solis^a, and James A. Dias^c

^aResearch Unit in Reproductive Medicine, Hospital de Ginec Obstetricia "Luis Castelazo Ayala", Instituto Mexicano del Seguro Social, México D.F., Mexico.

^b Department of Biochemistry, Faculty of Medicine, Universidad Nacional Autónoma de México, México D.F., México.

^c Wadsworth Center, David Axelrod Institute for Public Health, Albany NY, USA.

Abstract

The human (h) follicle-stimulating hormone receptor (FSHR) belongs to the superfamily of G protein-coupled receptors (GPCRs). This receptor consists of 695 amino acid residues and is preferentially coupled to the G_s protein. This receptor is highly conserved among species (overall homology, 85%), with a 25 %-69 % homology drop when compared to the human LH and TSH receptors. Although studies in prototypical rhodopsin/ β -adrenergic receptors suggest that multiple domains in the intracellular loops (iL) and the carboxyl-terminus (Ctail) of these receptors contribute to G protein coupling and receptor expression, there is a paucity of structure/function data on the role of these domains in FSHR function. Employing point mutations we have found that several residues present in the iL2 of the hFSHR are important for both coupling the receptor to the G_s protein and maintaining the receptor molecule in an inactive conformation. In fact, HEK-293 cells expressing several hFSHR mutants with substitutions at R⁴⁵⁰ (central to the highly conserved ERW triplet motif) and T⁴⁵³ (a potential target for phosphorylation) failed to mediate ligand-provoked G_s protein activation but not agonist binding, whereas substitutions at the hydrophobic L⁴⁶⁰ (a conserved residue present in all glycoprotein hormone receptors) conferred elevated basal cAMP to the transfected cells. Thus, this particular loop apparently acts as a conformational switch for allowing the receptor to adopt an active conformation upon agonist stimulation. Residues in both ends of the iL3 are important for signal transduction in a number of GPCRs, including the FSHR. We have recently explored the importance of the reversed BBXXB motif (BXXBB; where B represents a basic residue and X a non-basic residue) present in the juxtamembrane region of the hFSHR iL3. A hFSHR mutant with all basic amino acids present in the iL3 BXXBB motif replaced by alanine failed to bind agonist and activate effector, and was expressed as an immature =62 kDa form of the receptor. Individual substitutions of basic residues resulted in mutants that bound agonist normally but failed to activate effector when replaced at R⁵⁵² or R⁵⁵⁶. Triple mutations in the same motif located in the NH₂-end of the Ctail resulted in a complete inability of the receptor to bind agonist and activate effector, whereas individual substitutions resulted in decreased or virtually abolished agonist binding and cAMP accumulation, with both functions correlating with the detected levels of mature (80 kDa) forms of the receptor. Thus, the BXXBB motif at the iL3 of the FSHR is essential for coupling the activated receptor to the G_s protein, whereas the same motif in the Ctail is apparently more important for membrane expression. The role of cysteine residues present in the Ctail of the FSHR is an enigma since there are no conserved cysteines amongst LHR, FSHR and TSHR. C⁶²⁹ and C⁶⁵⁵ are conserved in the gonadotropin receptors but not in the TSHR. Alanine replacement of C⁶²⁷ had no effect on

*Corresponding author. Address: Research Unit, IMSS, Apartado Postal 99-065, Unidad Independencia, México 10101, D.F., México. FAX: +52-55-5616-2278. E-mail: aulloa@servidor.unam.mx

hFSHR expression and function, whereas the same mutation at C⁶²⁹ altered membrane expression and signal transduction. Serine or threonine substitutions of C⁶⁵⁵ did not modify any of the parameters analyzed. In the hFSHR, C⁶²⁹ may be a target for palmitoylation, and apparently it is the only cysteine residue in the Ctail domain that might play an important role in receptor function.

Keywords

Follicle-stimulating hormone receptor; Signal transduction; Receptor mutation

Introduction

Follicle-stimulating hormone (FSH) or follitropin belongs to a family of closely related glycoproteins composed of FSH, luteinizing hormone (LH) choriogonadotropin hormone (CG) and thyroid-stimulating hormone (TSH). This gonadotropin is produced by the anterior pituitary gland and co-regulates with LH the maintenance of several essential reproductive processes including gametogenesis, follicular development, and ovulation. FSH evokes its biological effects by interacting with a high affinity receptor (R) located on the plasma membrane of its target cells in the ovary and the testis. The FSHR is a glycoprotein that belongs to the superfamily of G protein-coupled receptors (GPCRs), specifically the family of rhodopsin-like receptors, which consist of a single polypeptide chain of variable length, that threads back and forth across the lipid bilayer seven times forming characteristic α -helical transmembrane-spanning domains (TM) connected by alternating extracellular and intracellular loops (iL), with an extracellular NH₂-terminal domain and an intracellular COOH-terminal segment (Ctail) (Ulloa-Aguirre and Conn, 1998; Lu et al., 2002). These receptors mediate their intracellular actions via activation of one or more guanine-nucleotide-binding signal transducing proteins (G proteins). The human FSHR consists of 695 amino acids (the first 17 amino acids encoding the signal sequence) (Simoni et al., 1997; Ulloa-Aguirre and Timossi 1998; Dias et al., 2002); upon activation by agonist, the activated receptor may trigger activation of a number of intracellular signaling pathways. In the classical, linear signaling cascade, occupancy of the FSHR causes activation of the heterotrimeric G_s protein, which in turn stimulates the effector adenylyl cyclase with the consequent increase in the synthesis of the second messenger cAMP, activation of PKA, phosphorylation of cAMP response element-binding protein, and activation of transcription (Reichert and Dattatreya Murty, 1989). Nevertheless, increasing evidence indicates that in addition to the adenylyl cyclase/cAMP/PKA signaling pathway, activation of the FSH receptor by its cognate ligand also triggers activation of other intracellular signaling cascades, including the MAPK and PI3-K/Akt pathways (Cameron et al., 1996; Maizels et al., 1998; González-Robayna et al., 2000; Richards 2002; Seger et al., 2001).

Site-directed mutagenesis and chimeric studies of several GPCRs belonging to the family of rhodopsin-like receptors indicate that several cytoplasmic domains of these receptors, particularly the iL2, the juxtamembrane portions of the iL3 and the Ctail, are involved in signal transduction (Ulloa-Aguirre and Conn 1998). In contrast to the related TSHR and LHR, there is a paucity of structure-function data on the role of the intracellular domains in FSHR-mediated signal transduction and receptor expression. Here we summarize some recent information on the role of the intracytoplasmic domains in function of the hFSHR.

Intracellular loop 1

This relatively short FSHR domain encompasses 13 amino acid residues [including those residues in the juxtamembrane portions of the TM1 and TM2 (Nakamura et al., 1998a, Ulloa-Aguirre and Timossi, 1998; Dias et al., 2002)] with 100% amino acid sequence homology between the human and the rat FSHRs. Replacement of all but one of the serine and threonine

residues present in the iL1 of the rat FSHR did not affect agonist induced cAMP accumulation but completely abolished agonist-induced, cAMP-independent protein kinase phosphorylation and impaired agonist-induced uncoupling and arrestin 3-associated receptor internalization (Nakamura et al., 1998a,b; Krishnamurthy et al., 2003). Apparently, these mutations not only impair iL1 phosphorylation but also result in a more generalized conformational change that prevents receptor phosphorylation. Interestingly, the triple rat FSHR iL1 mutation (T³⁶⁹I/S³⁷¹L and T³⁷⁶N) also resulted in constitutive activation of the receptor, whereas replacement of T³⁷⁰ (T³⁷⁸ in the hFSHR) prevented cell surface expression (Nakamura et al., 1998a). As noticed previously (Perez et al., 1996; Hwa et al., 1997; Nechamen and Dias, 2003; Ulloa-Aguirre et al., 2004), single mutations may result in the production of misfolded, trafficking-defective proteins or in constitutively activated conformers. In the case of the constitutively active rFSHR iL1 mutant, the structural alteration may disturb necessary interhelical and/or intracellular loop interactions required for dampening of basal activity.

Intracellular loop 2

The importance of the iL2 in signal transduction has been documented for a number of GPCRs (Ulloa-Aguirre and Conn, 1998). In fact, peptides mimicking the iL2 sequence of some GPCRs, may efficiently block receptor-G protein coupling (Varrault et al., 1994; Qian et al., 1998). In particular, the Glu/Asp-Arg-Tyr motif (E/DRY motif, permuted to ERW in the TSH, LH and FSH receptors) present in the majority of rhodopsin-like receptors has been implicated in G protein interaction and receptor activation (Gershergorn and Osman, 2001). Mutation of the central R has resulted in either partial or complete abolition of agonist-stimulated signal transduction (Chazenbalk et al., 1991; Franke et al., 1992; Rosenthal et al., 1993; Zhu et al., 1994; Jones et al., 1995; Acharya and Karnik, 1996; Arora et al., 1997; Seibold et al., 1998; Schultz et al., 1999) or in variable degrees of constitutive activation of the altered receptor, depending on the nature of the replacing amino acid residue (Fanelli et al., 1999; Scheer et al., 2000). Other residues, such as hydrophobic leucines located in the middle of the iL2 (Moro et al., 1993; Arora et al., 1995) and residues present in the carboxyl-terminal end (Okamoto and Nishimoto, 1992; McClue et al., 1994; Burnstein et al., 1998) (Fig. 1A) of several GPCRs, including the LH and TSH receptors (Chazenbalk et al., 1990; Schultz et al., 1999), have been also implicated in G protein activation.

Transient transfection of several plasmids containing a complementary cDNA construct encoding either the free Wt hFSHR iL2 or the free hFSHR iL2 mutated at R⁴⁵⁰, T⁴⁵³ [a potentially phosphorylable residue in the Wt rat FSHR (Nakamura et al., 1998b)] and/or L⁴⁶⁰ to human embryonic kidney 293 (HEK-293) cells stably expressing the hFSHR, resulted in distinct effects on FSH-stimulated intracellular signaling. Expression of the free Wt iL2 as well as the R⁴⁵⁰A, R⁴⁵⁰K and L⁴⁶⁰A free iL2 mutants led to a significant (~40-50%) attenuation in maximum FSH-stimulated cAMP accumulation, whereas expression of R⁴⁵⁰H, T⁴⁵³A and R⁴⁵⁰A/T⁴⁵³A/L⁴⁶⁰A free iL2 mutants did not affect FSH-provoked intracellular signaling, thus suggesting a specific role of iL2 residues in hFSHR function (Timossi et al., 2002). Further studies on modified hFSHRs corroborated the role of particular iL2 residues in receptor function. Agonist-stimulated cAMP production was virtually abolished by changing the hydrophilic R⁴⁵⁰ to A or H (a basically charged residue with a more rigid side chain) in the hFSHR, whereas the conservative substitution R? K did not affect FSH-stimulated intracellular signaling (Fig. 2A) (Timossi et al., 2002). None of the mutations introduced altered either cell surface membrane expression or ligand binding affinity of the modified hFSHR. These observations are in line with the results of studies on the related LH receptor, in which similar substitutions impaired the ability of the mutants to mediate a signaling response (Dhanawada et al., 1996; Schultz et al., 1999), indicating that the mechanisms underlying gonadotropin receptor activation through residues located in the amino-terminal end of the iL2 appear to be similar. Thus, a flexible, positively charged residue at this position is apparently required in

both gonadotropin receptors to allow the iL2 for proper interactions with other receptor domains that may yield an active receptor configuration upon activation by agonist. In agreement with studies on altered rat FSHRs (Nakamura et al., 1998b), replacement of T for A at position 453 of the hFSHR virtually abolished the ability of the mutated receptor to mediate FSH-provoked cAMP accumulation, despite proper membrane expression (Fig. 2B) (Timossi et al., 2002). It is possible that this particular residue may be important to form the active structure of the FSHR via interaction with other iL2 residues, as suggested for bovine rhodopsin (Yamashita et al., 2000) in which replacement of those NH₂-terminal iL2 amino acid residues expected to cluster on the same face forming the active structure of rhodopsin, prevented G protein activation.

The importance of the iL2 on FSHR activation is emphasized by studies on mutant hFSHRs bearing replacements at the conserved L⁴⁶⁰ residue; upon expression in HEK-293 cells, the L⁴⁶⁰D, L⁴⁶⁰P and L⁴⁶⁰A mutants exhibited variable degrees of constitutive activation and altered responses to agonist stimulation; replacement for K or R did not affect basal cAMP production but blunted or reduced the receptor response to further FSH stimulation (Fig. 3) (Timossi et al., 2002). Thus, it seems that disruption of the bonds stabilized by the hydrophobic L⁴⁶⁰ may either promote varying degrees of receptor isomerization to an active, unconstrained state or compromise receptor/G_s-protein activation, depending on the nature of the replacing amino acid residue. These findings strongly suggest that the iL2 of the FSHR may actually act as a conformational switch for allowing the receptor to adopt an active conformation upon agonist stimulation, rather than as a G protein recognition domain.

Intracellular loop 3

Several structural determinants present in this loop govern receptor cell surface residency and receptor-G protein interactions. In fact, this loop is considered pivotal in attenuation of agonist-stimulated, receptor-mediated intracellular signaling via internalization (Nakamura et al., 1998b; Krishnamurthy et al., 2003; Bhaskaran et al., 2003; Cohen et al., 2003). Naturally occurring point mutations and studies employing synthetic peptide strategies have provided some information on the potential role of this intracytoplasmic region in FSHR-mediated signal transduction. A point mutation involving the highly conserved basic residue R⁵⁵⁶ (Fig. 1B) led to decreased FSH-stimulated cAMP accumulation (Beau et al., 1998), while mutations in the conserved aspartate (D⁵⁵⁰) provoked constitutive activation of the hFSHR probably by disrupting the helix capping structure that presumably helps to maintain the receptor in an inactive conformation (Gromoll et al., 1996; Schultz et al., 2000; Haywood et al., 2002). Studies using synthetic peptides homologous to the rat FSHR iL3 showed that the peptide corresponding to residues 533 to 555 promoted guanine nucleotide exchange in testes light membranes and reduced FSH-stimulated cAMP production and estrogen production from primary Sertoli cell cultures (Grasso et al., 1995b); a more restricted peptide (residues 551 to 555) bearing the BBXXB motif reversed (BXXBB, where B is a basic amino acid) involved in G protein activation of several cell surface membrane receptors (Okamoto and Nishimoto, 1992; Wu et al., 1995; Pauwels et al., 1999, 2001; Cheung et al., 1991; Murthy and Makhoulf, 1999), provoked similar effects (Grasso et al., 1995a).

The role of the BXXBB motif present in the juxtamembrane region of the hFSHR on receptor function has been recently analyzed in more detail in our laboratories (Cohen et al., 2003; Timossi et al., 2004). Replacement of the three basic residues by alanine yielded a ~62 kDa under glycosylated form of the receptor, which was unable to bind agonist and activate effector (Figs. 4A and D). Nevertheless, the functional role of each basic residue appears to differ depending on its position within the minimal motif. As observed in the LH and TSH receptors (Chazenbalk et al., 1991; Schultz et al., 2000), replacement of K⁵⁵⁵ (corresponding to K⁵⁵² and K⁶²⁴ in the LH and TSH receptors) with alanine did not alter signal transduction (Fig. 4B).

Nevertheless, this particular hFSHR mutant exhibited an increased turnover probably due to increased ubiquitination of the altered receptor (Cohen et al., 2003; Timossi et al., 2004). In contrast, mutation of R⁵⁵² or R⁵⁵⁶ profoundly altered intracellular signaling (Fig. 4B) without affecting the ligand binding ability (Fig. 4E) or internalization rate of the receptor (Timossi et al., 2004). These latter effects clearly differ from the scenario prevailing in the other glycoprotein receptors since replacement of the basic K⁵⁶⁶ in the hLHR or K⁶²¹ in the hTSHR (corresponding to R⁵⁵² in the hFSHR), had no effect on agonist-provoked cAMP accumulation, whereas substitution of the comparable hFSHR R⁵⁵⁶ residue in these receptors led to either partial (in the hLHR) or complete (in the hTSHR) functional impairment (Chazenbalk et al., 1991; Schulz et al., 2000). Further, in the human FSH and TSH receptors, simultaneous substitution of all three basic residues completely abolished agonist-stimulated cAMP production and severely impaired intracellular trafficking and membrane expression of the modified receptors (Chazenbalk et al., 1990; Timossi et al., 2004), whereas in the rat LHR (92% homology with the human counterpart in the serpentine region of the receptor) simultaneous replacement of two out of the three cationic residues present in this motif had no functional consequence (Wang et al., 1993). Therefore, the ultimate basic residue of the BXXBB motif in all glycoprotein receptors appears to play a major role in the interaction of the receptor with the G_s protein. In the hFSHR, this motif may be one of the sites involved in receptor-G_s protein coupling (Strader et al., 1987; Cheung et al., 1992; Wu et al., 1995; Kohen et al., 2001) or alternatively it may be important to preserve the packing and interactions of the transmembrane domains 5 and 6 (Kudo et al., 1996), thereby favoring the accessibility of the G protein coupling sites or the formation of an active conformation upon agonist binding.

Carboxyl-terminal domain

Several structural and functional features characterize this particular hFSHR domain (Fig. 1C): *i*) The domain is rich in serine and threonine residues which may be potential sites for phosphorylation; nevertheless, studies on its rat counterpart have clearly shown that in contrast to the LHR, the Ctail of the FSHR is not phosphorylated (Nakamura et al., 1995a,b); *ii*) The hFSHR Ctail contains the minimal BBXXB motif reversed in its juxtamembrane region, whereas the rat receptor does not bear this motif but rather a BBXB motif [also involved in G protein coupling of other receptors (Okamoto and Nishimoto 1992)] located in the segment encompassed by residues 650-653. The last two residues of the BXXBB motif (R⁶¹⁷ and R⁶¹⁸) and the preceding F⁶¹⁶ constitute the NH₂ end of the highly conserved F(x)₆LL motif required for receptor transport to the cell membrane (Duvernay et al., 2004); *iii*) The domain exhibits at least 2 cysteine residues (at positions 627 and 629) which are potential sites for palmitoylation; however, evidence that this domain is esterified by palmitoylation is lacking. Two of the 3 cysteine residues present in the hFSHR (C⁶²⁹ and C⁶⁵⁵) are also present in the LHR but not in the TSHR (Fig. 1C).

The hFSHR Ctail-BXXBB motif appears to be more important for receptor expression than for G protein coupling, as suggested by studies in which the basic residues present in this motif were replaced by alanine (Timossi et al., 2004). Substitution of K⁶¹⁴ reduced both ligand binding (Fig. 4F) and membrane localization of the receptor (insert of Fig. 4B) to ~60% of the levels exhibited by the Wt receptor; consequently, cAMP production was also reduced (Fig. 4C). Meanwhile, replacement of R⁶¹⁷ or R⁶¹⁸ further reduced the ligand binding capacity and membrane expression of the mutant receptor, and virtually abolished FSH-stimulated cAMP production. Thus, mutations in these latter basic residues may provoke conformational changes in the NH₂-terminal end of the Ctail, which includes the above mentioned F(x)₆LL motif (F⁶¹⁶, L⁶²³, and L⁶²⁴), and thereby impair both receptor trafficking and cell membrane localization of the receptor.

Generation of a hFSHR Ctail structural model yielded a family of nine all- α helix globule-like models showing two different topologies that preserve the spatial arrangement of the helical structures. The predicted structures showed a bundle of α -helices connected by loop regions; as other all- α proteins, these α helices are packed pairwise against each other in an antiparallel fashion so that each helix provides the hydrophobic side chains for packing interactions (Branden and Tooze, 1991). The best-ranked model is composed of a three- α helices bundle connected by loop regions and a large loop of non-conventional secondary structure, and is completed by a small α helix non packed to the other α helices (Fig. 5). The region of non-conventional structure (residues 648 to 670) was also predicted to be a natively disordered region by the program DISOPRED2 (Jones and Ward, 2003; Ward et al., 2004). These natively unstructured regions have been described in many binding, assembler, scavenger and regulatory proteins (Tomba, 2002). Thus, this region in the hFSHR may participate in G protein recognition and binding. In nine of the models, included the best-ranked, the loop containing Cys⁶²⁹ is in close contact with the loop containing Cys⁶⁵⁵. Although the sulfur atom of Cys⁶²⁹ is at only 3.08 Å from the 2H β atom of Cys⁶⁵⁵, the formation of a disulfide bond between these cysteine residues would be otherwise extremely rare due to the reducing environment inside cells. Nevertheless, cysteine residues may play a key structural role within the intracellular environment; in GPCRs, cysteines in the Ctail are particularly important since they are potential sites for receptor palmitoylation. Among some other functions, this lipid modification appears necessary to form a fourth cytoplasmic loop (Ulloa-Aguirre and Conn, 1998). In some GPCRs, such as the β_2 -adrenergic receptor, mutation of the palmitoylated cysteine leads to greatly decreased interaction of the mutant receptor with the G_s protein (O'Dowd et al., 1989), whereas in others it has little or no effect in receptor function (van Koppen et al., 1991; Kennedy et al., 1993). As the TSH and LH receptors, the hFSHR exhibits highly conserved cysteine residues with a neighboring basic residue (K⁷⁰⁰, K⁶⁴⁵ and K⁶²⁶ in the hTSHR, hLHR and hFSHR, respectively) that appears to be essential for palmitoylation (Belanger et al., 2001). In fact, it has been shown that the human and rat TSHRs as well as the rat LHR are palmitoylated in their Ctail and that mutation of their corresponding palmitoylated cysteines results in either abnormal trafficking or intracellular trapping of the mutant receptor (Zhu et al., 1995; Kosugi and Mori, 1996; Tanaka et al., 1998). In the hFSHR, replacement of Cys⁶²⁷ with alanine did not alter receptor function, agonist binding or membrane surface expression, whereas alanine substitution at Cys⁶²⁹ or at both Cys⁶²⁷ and Cys⁶²⁹ decreased FSH-stimulated cAMP accumulation by 20 to 30% (Fig. 6), and impaired both agonist binding and receptor expression (not shown). Meanwhile, replacement of Cys⁶⁵⁵ by alanine but not by threonine or serine, decreased agonist-stimulated cAMP production without altering cell surface membrane expression. These results suggests that only Cys⁶²⁹ plays an important role in hFSHR function and that as shown for the LHR (Zhu et al., 1995), this particular residue may be involved in receptor palmitoylation and cell surface membrane expression.

Conclusions

We have summarized herein some recent information on the role of the intracytoplasmic domains of the hFSHR on G protein coupling and receptor expression. Although the hFSHR preferentially couples to the G_s α -subunit, there is some experimental evidence suggesting that the FSHR may additionally signal through the pertussis toxin-sensitive G_{i/o}-mediated pathways (Eskola et al., 1994; Arey et al., 1997; Timossi et al., 1998). In this regard, alternative spliced variants of the receptor may be one of the mechanisms by which particular intracytoplasmic domains of the FSHR may signal through these G proteins (Sairam et al., 1996). As mentioned above, the FSHR also signals through cAMP- dependent, but PKA-independent alternate signaling cascades (Richards et al., 2002); in addition, it has been shown that the adapter protein 14-3-3 τ , a member of the 14-3-3 protein family which play a key role in signal transduction pathways, cell division and apoptosis (Tzivion and Avruch, 2002), interacts with the iL2 of the hFSHR, suggesting a role for this cytoplasmic protein in FSH-mediated cAMP independent

signaling (Cohen et al., 2004). APPL1, another adapter protein that interacts with the p110 α catalytic subunit of PI3K and with inactive Akt (Mitsuuchi et al., 1999), has been more recently identified as an hFSHR iL1-interacting partner, providing a potential link between the FSHR and the PI3K/Akt signaling pathway (Nechamen et al., 2004). One thus may envision a complex system of multiple, pleiotropic signals triggered by the activated FSHR, in which compartmentalization and oligomerization of particular receptor populations may potentially play fundamental roles.

Acknowledgments

The studies from the authors' laboratories have been supported by grants from the Consejo Nacional de Ciencia y Tecnología (CONACyT, Mexico) (grant 38056M), the Fondo para el Fomento de la Investigación (FOFOI)-Instituto Mexicano del Seguro Social, México (grant 2004/117) (to A.U.-A.), and the National Institutes of Health (Bethesda, MD, USA) (grant NIH-HD18407) (to J.A.D)

References

- Acharya S, Karnik SS. Modulation of GDP release from transducin by the conserved Glu¹³⁴-Arg¹³⁵ sequence in rhodopsin. *J. Biol. Chem* 1996;271:25406–25411. [PubMed: 8810308]
- Arey BJ, Stevis PE, Deecher DC, Shen ES, Frail DE, Negro-Vilar A, Lopez FJ. Induction of promiscuous G protein coupling of the follicle-stimulating hormone (FSH) receptor: a novel mechanism for transducing pleiotropic actions of FSH isoforms. *Mol. Endocrinol* 1997;11:517–526. [PubMed: 9139796]
- Arora KK, Cheng Z, Catt KJ. Mutations of the conserved DRS motif in the second intracellular loop of the gonadotropin-releasing hormone receptor affect expression, activation, and internalization. *Mol. Endocrinol* 1997;11:1203–1212. [PubMed: 9259312]
- Arora KK, Sakai A, Catt KJ. Effects of second intracellular loop mutations on signal transduction and internalization of the gonadotropin-releasing hormone receptor. *J. Biol. Chem* 1995;270:22820–22826. [PubMed: 7559413]
- Ballesteros JA, Shi L, Javitch JA. Structural mimicry in G protein-coupled receptors: implications of the high-resolution structure of rhodopsin for structure-function analysis of rhodopsin-like receptors. *Mol. Pharmacol* 2001;60:1–19. [PubMed: 11408595]
- Beau I, Touraine P, Meduri G, Gougeon A, Desroches A, Matuchansky C, Milgrom E, Misrahi M. A novel phenotype related to partial loss of function mutations of the follicle-stimulating hormone receptor. *J. Clin. Invest* 1998;102:1352–1359. [PubMed: 9769327]
- Belanger C, Ansanay H, Qanbar R, Bouvier M. Primary sequence requirements for S-acylation of β_2 -adrenergic receptor peptides. *FEBS Lett* 2001;499:59–64. [PubMed: 11418112]
- Bhaskaran RS, Min L, Krishnamurthy H, Ascoli M. Studies with chimeras of the gonadotropin receptors reveal the importance of third intracellular loop threonines on the formation of the receptor/nonvisual arrestin complex. *Biochemistry* 2003;42:13950–13958. [PubMed: 14636063]
- Bradley P, Chivian D, Meiler J, Misura KM, Rohl CA, Schief WR, Wedemeyer WJ, Schueler-Furman O, Murphy P, Schonbrun J, Strauss CE, Baker D. Rosetta predictions in CASP5: successes, failures, and prospects for complete automation. *Proteins* 2003;53:457–468. [PubMed: 14579334]
- Branden, C.; Tooze, J. Introduction to protein structure. Garland Publishing, Inc.; New York: 1991. p. 33-40.
- Burnstein ES, Spalding TA, Brann MR. The second intracellular loop of the m5 muscarinic receptor is the switch which enables G-protein coupling. *J. Biol. Chem* 1998;273:24322–24327. [PubMed: 9733718]
- Cameron MR, Foster J.S., Bukovsky A, Wimalasena J. Activation of mitogen-activated protein kinases by gonadotropins and cyclic adenosine 5'-monophosphates in porcine granulosa cells. *Biol. Reprod* 1996;55:111–119. [PubMed: 8793065]
- Chazenbalk GD, Nagayama Y, Wadsworth H, Russo D, Rapoport B. Signal transduction by the human thyrotropin receptor: studies on the role of individual amino acid residues in the carboxyl terminal region of the third cytoplasmic loop. *Mol. Endocrinol* 1991;5:1523–1526. [PubMed: 1663578]

- Cheung AH, Huang RR, Graziano MP, Strader CD. Specific activation of Gs by synthetic peptides corresponding to an intracellular loop of the β -adrenergic receptor. *FEBS Lett* 1991;279:277–280. [PubMed: 1848192]
- Cohen BD, Bariteau JT, Magenis LM, Dias JA. Regulation of follitropin receptor cell surface residency by the ubiquitin-proteasome pathway. *Endocrinology* 2003;144:4393–4402. [PubMed: 12960054]
- Cohen BD, Nechamen Ch. A. Dias JA. Human follitropin receptor (FSHR) interacts with the adapter protein 14-3-3 τ . *Mol. Cell. Endocrinol* 2004;220:1–7. [PubMed: 15196694]
- Dhanawada KR, Vijapurkar U, Ascoli M. Two mutations of the lutropin/choriogonadotropin receptor that impair signal transduction also interfere with receptor-mediated endocytosis. *Mol. Endocrinol* 1996;10:544–554. [PubMed: 8732685]
- Dias JA, Cohen BD, Lindau-Shepard B, Nechmen Ch.A. Peterson AJ, Schmidt A. Molecular, structural, and cellular biology of follitropin and follitropin receptor. *Vitam. Horm* 2002;64:249–322. [PubMed: 11898394]
- Duvernay MT, Zhou F, Wu G. A conserved motif for the transport of G protein-coupled receptors from the endoplasmic reticulum to the cell surface. *J. Biol. Chem* 2004;279:30741–30750. [PubMed: 15123661]
- Eskola V, Rannikko A, Huhtaniemi I, Warre DW. Ontogeny of the inhibitory guanine nucleotide-binding regulatory protein in the rat testis: mRNA expression and modulation by LH and FSH action. *Mol. Cell. Endocrinol* 1994;102:63–68. [PubMed: 7926274]
- Fanelli F, Barbier P, Zanchetta D, De Benedetti PD, Chini B. Activation mechanism of human oxytocin receptor: a combined study of experimental and computer-simulated mutagenesis. *Mol. Endocrinol* 1999;13:214–225.
- Franke RR, Sakmar TP, Graham RM, Khorana HG. Structure and function in rhodopsin. Studies of the interaction between the rhodopsin cytoplasmic domain and transducin. *J. Biol. Chem* 1992;267:14767–14774. [PubMed: 1634520]
- Gershengorn MC, Osman R. Insights into G protein-coupled receptor function using molecular models. *Endocrinology* 2001;142:2–10. [PubMed: 11145559]
- Gonzalez-Robayna JJ, Falender AE, Ochsner S, Firestone GL, Richards JS. Follicle-Stimulating hormone (FSH) stimulates phosphorylation and activation of protein kinase B (PKB/Akt) and serum and glucocorticoid-induced kinase (Sgk): evidence for a kinase-independent signaling by FSH in granulosa cells. *Mol. Endocrinol* 2000;14:1283–300. [PubMed: 10935551]
- Grasso P, Deziel MR, Reichert LE Jr. Synthetic peptides corresponding to residues 551 to 555 and 650 to 653 of the rat testicular follicle-stimulating hormone (FSH) receptor are sufficient for post-receptor modulation of Sertoli cell responsiveness to FSH stimulation. *Regul. Pept* 1995a;60:177–183. [PubMed: 8746544]
- Grasso P, Leng N, Reichert LE Jr. A synthetic peptide corresponding to the third cytoplasmic loop (residues 533 to 555) of the testicular follicle-stimulating hormone receptor affects signal transduction in rat testis membranes and in intact cultured rat Sertoli cells. *Mol. Cell. Endocrinol* 1995b;110:35–41. [PubMed: 7672451]
- Gromoll J, Simoni M, Nieschlag E. An activating mutation of the follicle-stimulating hormone receptor autonomously sustains spermatogenesis in a hypophysectomized man. *J. Clin. Endocrinol. Metab* 1996;81:1367–1370. [PubMed: 8636335]
- Haywood M, Tymchenko N, Spaliviero J, Koch A, Jimenez M, Gromoll J, Simoni M, Nordhoff V, Handelsman DJ, Allan CM. An activated human follicle-stimulating hormone (FSH) receptor stimulates FSH-like activity in gonadotropin-deficient transgenic mice. *Mol. Endocrinol* 2002;16:2582–2591. [PubMed: 12403847]
- Hwa J, Gaivin R, Porter JE, Perez DM. Synergism of constitutive activity in α 1-adrenergic receptor activation. *Biochem. J* 1997;36:633–639.
- Jones DT, Ward JJ. Prediction of disordered regions in proteins from position specific scoring matrices. *Proteins* 2003;53:573–578. [PubMed: 14579348]
- Jones PG, Curtis CA, Hulme EC. The function of a highly-conserved arginine residue in activation of the muscarinic M1 receptor. *Eur. J. Pharmacol* 1995;288:251–257. [PubMed: 7774669]
- Kennedy ME, Limbird LE. Mutations of the α 2A-adrenergic receptor that eliminate detectable palmitoylation do not perturb receptor-G-protein coupling. *J. Biol. Chem* 1993;268:9003–8011.

- Kim DE, Chivian D, Baker D. Protein structure prediction and analysis using the Robetta server. *Nucleic Acids Res* 2004;32:W526–W531. [PubMed: 15215442]
- Kohen R, Fashingbauer LA, Heidmann DE, Guthrie CR, Hamblin MW. Cloning of the mouse 5-HT₆ serotonin receptor and mutagenesis studies of the third cytoplasmic loop. *Mol. Brain Res* 2001;90:110–117. [PubMed: 11406289]
- Kosugi S, Mori T. Cysteine-699, a possible palmitoylation site of the thyrotropin receptor, is not crucial for cAMP or phosphoinositide signaling but is necessary for full surface expression. *Biochem. Biophys. Res. Commun* 1996;221:636–640. [PubMed: 8630013]
- Krishnamurthy H, Galet C, Ascoli M. The association of arrestin-3 with the follitropin receptor depends on receptor activation and phosphorylation. *Mol. Cell. Endocrinol* 2003;204:127–140. [PubMed: 12850288]
- Kudo M, Osuga Y, Kobilka BK, Hsueh AJW. Transmembrane regions V and VI of the human luteinizing hormone receptor are required for constitutive activation by a mutation in the third intracellular loop. *J. Biol. Chem* 1996;271:22470–22478. [PubMed: 8798412]
- Lu Z-L, Saldanha JW, Hulme EC. Seven-transmembrane receptors: crystals clarify. *Trends Pharmacol. Sci* 2002;23:140–146. [PubMed: 11879682]
- Maizels ET, Cottom J, Jones JC, Hunzicker-Dunn M. Follicle stimulating hormone (FSH) activates the p38 mitogen-activated protein kinase pathway, inducing small heat shock protein phosphorylation and cell rounding in immature rat ovarian granulosa cells. *Endocrinology* 1998;139:3353–3356. [PubMed: 9645711]
- McClue SJ, Baron BM, Harris BA. Activation of G_i protein by peptide of the muscarinic M₂ receptor second intracellular loop. *Eur. J. Pharmacol* 1994;267:185–193. [PubMed: 8050479]
- Mitsuuchi Y, Johnson SW, Sonoda G, Tanno S, Golemis EA, Testa JR. Identification of a chromosome 3p14.3-21.1 gene, APPL, encoding an adaptor molecule that interacts with the oncoprotein-serine/threonine kinase AKT2. *Oncogene* 1999;18:4891–4898. [PubMed: 10490823]
- Moro O, Lamah J, Högger P, Sadée W. Hydrophobic amino acid in the i2 loop plays a key role in receptor-G protein coupling. *J. Biol. Chem* 1993;268:22273–76. [PubMed: 8226735]
- Murthy KS, Makhoul GM. Identification of the G protein-activating domain of the natriuretic peptide clearance receptor (NPR-C). *J. Biol. Chem* 1999;274:17587–17592. [PubMed: 10364194]
- Nakamura K, Hipkin RW, Ascoli M. The agonist-induced phosphorylation of the rat follitropin receptor maps to the first and third intracellular loops. *Mol. Endocrinol* 1998a;12:580–591. [PubMed: 9544993]
- Nakamura K, Krupnick JG, Benovic JL, Ascoli M. Signaling and phosphorylation-impaired mutants of the rat follitropin receptor reveal an activation- and phosphorylation-independent but arrestin-dependent pathway for internalization. *J. Biol. Chem* 1998b;273:24346–24354. [PubMed: 9733722]
- Nechamen, Ch. A.; Thomas, RM.; Cohen, BD.; Acevedo, G.; Poulikakos, PI.; Testa, JR.; Dias, JA. Human follicle-stimulating hormone (FSH) receptor interacts with the adaptor protein APPL1 in HEK 293 cells: potential involvement of the PI3K pathway in FSH signaling. *Biol. Reprod* 2004;71:629–636. [PubMed: 15070827]
- Nechamen, Ch. A.; Dias, JA. Point mutations in follitropin receptor result in ER retention. *Mol. Cell. Endocrinol* 2003;201:123–131. [PubMed: 12706300]
- O'Dowd BF, Hnatowich M, Caron MG, Lefkowitz RJ, Bouvier M. Palmitoylation of the human β_2 -adrenergic receptor. Mutation of cys341 in the carboxyl tail leads to an uncoupled nonpalmitoylated form of the receptor. *J. Biol. Chem* 1989;264:7564–7569. [PubMed: 2540197]
- Okamoto T, Nishimoto I. Detection of G protein-activator regions in M₄ subtype muscarinic, cholinergic, and α_2 -adrenergic receptors based upon characteristics in primary structure. *J. Biol. Chem* 1992;267:8342–8346. [PubMed: 1314825]
- Pauwels PJ, Tardif S, Colpaert FC. Differential signalling of both wild-type and Thr³⁴³Arg dopamine D_{2short} receptor by partial agonists in a G-protein-dependent manner. *Biochem. Pharmacol* 2001;62:723–732. [PubMed: 11551517]
- Perez DM, Hwa J, Gaivin R, Mathur M, Brown F, Graham RM. Constitutive activation of a single effector pathways: evidence for multiple activation states of a G protein-coupled receptor. *Mol. Pharmacol* 1996;49:112–122. [PubMed: 8569696]

- Qian A, Wang W, Sanborn BM. Evidence for the involvement of several intracellular domains in the coupling of oxytocin receptor to Gαq/11. *Cell. Signal* 1998;10:101–105. [PubMed: 9481484]
- Reichert LE, Dattatreya Murthy B. The follicle-stimulating hormone (FSH) receptor in testis: interaction with FSH, mechanism of signal transduction, and properties of the purified receptor. *Biol. Reprod* 1989;40:13–26. [PubMed: 2493820]
- Richards JS, Russell DL, Ochsner S, Hsieh M, Doyle KH, Falender AE, Lo YK, Sharma SC. Novel signaling pathways that control ovarian follicular development, ovulation, and luteinization. *Recent Prog. Horm. Res* 2002;57:195–220. [PubMed: 12017544]
- Rosenthal W, Antaramian A, Gilbert S, Birnbaumer M. Nephrogenic diabetes insipidus. A V2 vasopressin receptor unable to stimulate adenylyl cyclase. *J. Biol. Chem* 1993;268:130303–13033.
- Sairam MR, Jiang LG, Yarney TA, Khan H. Alternative splicing converts the G-protein coupled follitropin receptor gene into a growth factor type I receptor: implication for pleiotropic actions of the hormone. *Mol. Reprod. Dev* 1997;48:471–479. [PubMed: 9364441]
- Scheer A, Costa T, Fanelly F, Benedetti PG, Mhaouty-Kodja S, Abuin L, Nenniger-Tosato M, Cotecchia S. Mutational analysis of the highly conserved arginine within the Glu/Asp-Arg-Tyr motif of the α_{1b}-adrenergic receptor: effects on receptor isomerization and activation. *Mol. Pharmacol* 2000;57:219–231. [PubMed: 10648631]
- Schulz A, Bruns K, Henklein P, Krause G, Schubert M, Gudermann T, Wray V, Schultz G, Schöneberg T. Requirement of specific intrahelical interactions for stabilizing the inactive conformation of glycoprotein hormone receptors. *J. Biol. Chem* 2000;275:37860–37869. [PubMed: 10967122]
- Schulz A, Schöneberg T, Paschke R, Shultz G, Gudermann T. Role of the third intracellular loop for the activation of gonadotropin receptors. *Mol. Endocrinol* 1999;13:181–190. [PubMed: 9973249]
- Seger R, Hanoch T, Rosenberg R, Dantes A, Merz WE, Strauss JF 3rd, Amsterdam A. The ERK signaling cascade inhibits gonadotropin-stimulated steroidogenesis. *J. Biol. Chem* 2001;276:13957–13964. [PubMed: 11278318]
- Seibold A, Dagarag M, Birnbaumer M. Mutations of the DRY motif that preserve β₂-adrenoceptor coupling. *Receptor Channels* 1998;5:375–385.
- Simoni M, Gromoll J, Nieschlag E. The follicle-stimulating hormone receptor: biochemistry, molecular biology, physiology, and pathophysiology. *Endocr. Rev* 1997;18:739–773. [PubMed: 9408742]
- Simons KT, Kooperberg C, Huang E, Baker D. Assembly of protein tertiary structures from fragments with similar local sequences using simulated annealing and Bayesian scoring functions. *J. Mol. Biol* 1997;268:209–225. [PubMed: 9149153]
- Simons KT, Ruczinski I, Kooperberg C, Fox BA, Bystroff C, Baker D. Improved recognition of native-like protein structures using a combination of sequence-dependent features of proteins. *Proteins* 1999;34:82–95. [PubMed: 10336385]
- Strader CD, Dixon RA, Cheung AH, Candelore MR, Blake AD, Sigal IS. Mutations that uncouple the β-adrenergic receptor from Gs and increase agonist affinity. *J. Biol. Chem* 1987;262:16439–16433. [PubMed: 2890637]
- Tanaka K, Nagayama Y, Nishihara E, Namba H, Yamashita S, Niwa M. Palmitoylation of human thyrotropin receptor: slower intracellular trafficking of the palmitoylation-defective mutant. *Endocrinology* 1998;139:803–806. [PubMed: 9449658]
- Timossi C, Maldonado D, Vizcaíno 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_s protein activation. *Mol. Cell. Endocrinol* 2002;189:157–168. [PubMed: 12039074]
- Timossi C, Barrios-de-Tomasi J, Zambrano E, González R, Ulloa-Aguirre A. A naturally occurring basically charged human follicle-stimulating hormone (FSH) variant inhibits FSH-induced androgen aromatization and tissue-type plasminogen activator enzyme activity in vitro. *Neuroendocrinol* 1998;67:153–163.
- Timossi C, Ortiz-Maldonado C, Pineda DB, Dias JA, Conn PM, Ulloa-Aguirre A. functional significance of the BBXXB motif reversed present in the cytoplasmic domains of the human follicle-stimulating hormone receptor. *Mol. Cell. Endocrinol* 2004;223:17–26. [PubMed: 15279907]
- Tomba P. Intrinsically unstructured proteins. *Trends Biochem. Sci* 2002;27:527–533. [PubMed: 12368089]

- Tzivion G, Avruch J. 14-3-3 Proteins: active cofactors in cellular regulation by serine/threonine phosphorylation. *J. Biol. Chem* 2002;277:3061–3064. [PubMed: 11709560]
- Ulloa-Aguirre, A.; Conn, PM. G protein-coupled receptors and the G protein family. In: Conn, PM., editor. *Handbook of Physiology-Endocrinology, Section 7. Cellular Endocrinology*. Oxford University Press; New York: 1998. p. 87-124.
- Ulloa-Aguirre A, Janovick JA, Brothers SP, Conn PM. Pharmacologic rescue of conformationally-defective proteins: implications for the treatment of human disease. *Traffic* 2004;5:821–837. [PubMed: 15479448]
- Ulloa-Aguirre A, Timossi C. Structure-function relationship of follicle-stimulating hormone and its receptor. *Hum. Reprod* 1998;4:260–283.Update
- van Koppen CJ, Nathanson NM. The cysteine residue in the carboxyl-terminal domain of the m2 muscarinic acetylcholine receptor is not required for receptor-mediated inhibition of adenylate cyclase. *J. Neurochem* 1991;57:1873–1877. [PubMed: 1658226]
- Varraut A, Le Nguyen D, McClue S, Harris B, Jouin P, Bockaert J. 5-hydroxytryptamine 1A receptor synthetic peptides. Mechanisms of adenylyl cyclase inhibition. *J. Biol. Chem* 1994;269:16720–16725. [PubMed: 8206993]
- Wang H, Jaquette J, Collison K, Segaloff DL. Positive charges in a putative amphiphilic helix in the carboxyl-terminal region of the third intracellular loop of the luteinizing hormone/chorionic gonadotropin receptor are not required for hormone-stimulated cAMP production but are necessary for expression of the receptor at the plasma membrane. *Mol. Endocrinol* 1993;7:1437–1444. [PubMed: 8114758]
- Ward JJ, Sodhi JS, McGuffin LJ, Buxton BF, Jones DT. Prediction and functional analysis of native disorder in proteins from the three kingdoms of life. *J. Mol. Biol* 2004;337:635–645. [PubMed: 15019783]
- Wu D, Jiang H, Simon ML. Differential $\alpha 1$ -adrenergic receptor sequences required for activating different $G\alpha$ subunits of Gq class of G proteins. *J. Biol. Chem* 1995;270:9828–9832. [PubMed: 7730363]
- Yamashita T, Terakita A, Shichida Y. Distinct roles of the second and third cytoplasmic loops of bovine rhodopsin in G protein activation. *J. Biol. Chem* 2000;275:34272–34279. [PubMed: 10930404]
- Zhu H, Wang H, Ascoli M. The lutropin/choriogonadotropin receptor is palmitoylated at intracellular cysteine residues. *Mol. Endocrinol* 1995;9:141–150. [PubMed: 7776964]
- Zhu SZ, Wang SZ, Hu J, El-Fakahany WW. An arginine residue conserved in most G protein-coupled receptors is essential for the function of the M1 muscarinic receptor. *Mol. Pharmacol* 1994;45:517–523. [PubMed: 8145736]

Figure 1.

Schematic of the second (iL2) (A) and third (iL3) (B) intracellular loops, and the carboxyl-terminus (Ctail) (C) of the human FSH receptor. The sequence alignment of the corresponding domains in other glycoprotein hormone receptors is also shown; conserved amino acids analyzed in the human FSHR are shaded in grey. Brackets in the carboxyl-terminal end of the iL3 and the amino-terminal end of the Ctail (B and C) delimit the BBXXB motif reversed which has been shown to be associated with G protein activation in other G protein-coupled receptors (Okamoto and Nishimoto, 1992). The square in the amino-terminal end of the Ctail delimits the F(x)₆LL motif required for receptor transport to the cell surface membrane (Duvernay et al., 2004). Figure 1A is reproduced from Timossi et al. (2002), with permission from Elsevier Ireland Ltd.

Figure 2.

Concentration-dependent FSH-stimulated cAMP accumulation (minus basal cAMP concentrations) in HEK-293 cells expressing the Wt and the R⁴⁵⁰A, R⁴⁵⁰K, R⁴⁵⁰H (A), and T⁴⁵³A (B) mutant hFSHRs. The inset shows the relative binding of the mutant hFSHR receptors. Reproduced from Timossi et al. (2002), with permission from Elsevier Ireland Ltd.

Figure 3.

A: Concentration-dependent FSH-stimulated cAMP accumulation in HEK-293 cells expressing the Wt and the L⁴⁶⁰A constitutively active hFSHR; bars: basal cAMP production. B: Basal cAMP accumulation in HEK-293 expressing the Wt and the L⁴⁶⁰D, L⁴⁶⁰P, L⁴⁶⁰K, L⁴⁶⁰R, and L⁴⁶⁰A mutant hFSHRs. Black-filled bars: constitutively active mutants. *P < 0.05 vs. Wt. C: FSH-stimulated cAMP accumulation (minus basal concentrations) in HEK-293 cells expressing the Wt and the L⁴⁶⁰ mutated hFSHRs. The inset shows the relative binding of the mutant hFSHR receptors. The results are representative of three separate experiments (A and C) or represent the mean $\pm$ SD of three independent experiments (B and inset). Reproduced from Timossi et al. (2002), with permission from Elsevier Ireland Ltd.

Figure 4.

Concentration-dependent basal and FSH-stimulated cAMP accumulation (A), specific [¹²⁵I]-labeled FSH binding (D) and Western blot analysis of hFSHR expression (inset) in HEK-293 cells expressing the Wt hFSHR and the mutant hFSHR in which all basic residues present in the BXXBB motif at the iL3 (iL3-AXXAA) or the Ctail (Ctail-AXXAA) were replaced with alanine. B and E show the functional analysis of HEK-293 cells expressing the Wt hFSHR or the iL3 hFSHR mutants in which R⁵⁵² (iL3-AXXBB), K⁵⁵⁵ (iL3-BXXAB) or R⁵⁵⁶ (iL3-BXXBA) were replaced with alanine. C and F show the concentration-dependent basal and FSH-stimulated cAMP accumulation, specific agonist binding, and Western blot analysis of hFSHR expression (inset) in HEK-293 cells expressing either the Wt hFSHR or the Ctail hFSHR mutants in which K⁶¹⁴ (Ctail-AXXBB), R⁶¹⁷ (Ctail-BXXAB) or R⁶¹⁸ (Ctail-BXXBA) were replaced with alanine. A-C are representative of 3-4 independent experiments, whereas D-F are the mean $\pm$ S.E.M. from three experiments in triplicate incubations. Reproduced from Timossi et al. (2004), with permission from Elsevier Ireland Ltd.

Figure 5.

Computational model of the hFSHR Ctail as disclosed by *de novo* modeling. The model was generated on Robetta server (Kim et al., 2004), which is based on the Rosetta software for protein prediction (Bradley et al., 2003; Simons et al., 1997, 1999). Robetta generates three- and nine-residue fragment libraries representing local conformations of amino acids as found in the RCSB Protein Data Bank. This library is used to assemble models by fragment insertion using a scoring function. The program eliminates the models showing too many local contacts and unlikely strand topologies, producing the best ranking models based on its scoring function. These models are finally compared with chains in the protein data bank to produce a Z-score, which represents the probability of getting a similar-length match by chance. The side chains of the Ctail hFSHR models were repacked using a Monte Carlo algorithm based on a backbone-dependent side-chain rotamer library. The best-ranked model, with the three cysteine residues (Cys⁶²⁷, Cys⁶²⁹ and Cys⁶⁵⁵) in color sticks is shown.

Figure 6. Concentration-dependent basal and FSH-stimulated cAMP production (representative of 3 independent experiments), and percent maximal cAMP production (mean $\pm$ S.E.M. from 3 experiments) in cells transiently expressing the Wt hFSHR or the Ctail-cysteine mutant hFSHRs.