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Review

Common and differential mechanisms of gonadotropin receptors

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Abstract. The gonadotropin receptors are G-protein-coupled receptors with unique structural and functional features, consisting of two halves. The N-terminal extracellular half (exodomain) binds the hormones, whereas the C-terminal membrane-associated half (endodomain) is responsible for receptor activation. In this review, the novel ternary interactions, contact points and mutual modulations among the exodomain, endodomain and hormone for hormone binding and signal generation are described based on the latest observations. This discussion is contrary to the view that the exodomain and endodomain are independent, at least functionally, and provides new insights into the receptor mechanisms for the gonadotropins and other G-protein-coupled receptors.

Key words. Receptor activation; exodomain; endodomain; signal transduction; luteinizing hormone; follicle-stimulating hormone; human chorionic gonadotropin.

Introduction

Luteinizing hormone (LH), human chorionic gonadotropin (hCG) and follicle-stimulating hormone (FSH) are gonadotropins [1]. FSH and LH play crucial roles in development of gonads for both sexes and regulation of the ovulation cycle in female mammals [2, 3], whereas hCG is involved in the maintenance of the ovarian corpora lutea in humans and, therefore, in pregnancy. Mutations of the receptors result in hereditary reproductive disorders [4]. Therefore, these hormones and their receptors have been targets for the treatment of fertility and infertility disorders [5, 6]. In addition, they are also implicated in ovarian and testicular cancers. LH and hCG bind to the LH receptor (LHR), whereas FSH binds to the FSH receptor (FSHR).

The FSHR and LHR comprise two equal halves, an extracellular N-terminal half (exodomain) and a membrane-associated C-terminal half (endodomain) as shown in figure 1 A. The exodomain is ~350 amino acids long, and is alone capable of high-affinity hormone binding [7–9] with hormone selectivity [10–12] but without hormone action [9, 13]. The membrane-associated C-terminal endodomain consists of seven transmembrane domains (TMs), three exoloops, three cytoloops, a C-terminal tail and a short extracellular extension connected TM1. Receptor activation occurs in the endodomain [14], which is structurally equivalent to the entire molecule of many other G-protein-coupled receptors [15]. Existing data suggest that glycoprotein hormones initially bind to the exodomain, and the resulting hormone/exodomain complex undergoes conformational adjustments [16] and modulates the endodomain to generate hormone signals [14, 15, 17]. The receptors are capable of activating two different effectors, adenylyl cyclase to produce cAMP, a second messenger, from ATP, and phospholipase Cβ to produce another second two messengers, diacyl glycerol and inositol phosphates. The two enzymes are activated by different mechanisms, and therefore the receptors generate two distinct signals [18].

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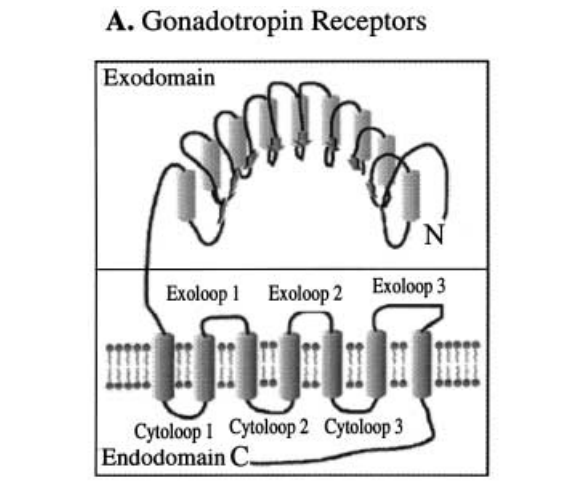

B. FSHR Leu Rich Repeats

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 29 AIELRFVLTK LRVIQKG AFSGFGD
 55 LEKIEISQNDVLEVIEAD VFSNLPK
 80 LHEIRIEKANNLLYINPE AFQNLPN
105 LQYLLISNTG IKHLPDVHKIHSLQK
130 V LLDIQDNINIHTIERN SFVGLSFES
156 V ILWLNKNG IQEIHNC AFNGTQ
178 LDELNLSDNNNLEELPND VFHGASGP
204 V ILDISRTRIHSLPSYGLENLKKLRA
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C. LHR Leu Rich Repeats

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 26 LARLSLAYLP VKVIPSQ  AFRGLNE
 50 VIKIEISQIDSLERIEAN  AFDNLLN
 75 LSEILIQNTKNLRYIEPG  AFINLPR
100 LKYLSICNTG IRKFPDVTKVFSSES
125 NFILEICDNLHITTIPGN  AFQGMNNE
151 SVTLKLYGNG FEEVQSH  AFNGTT
174 LTSLELKENVHLEKMHNG  AFRGATG
199 PKTLDISSTK LQALPSY  GLESIQR
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Figure 1. Structure and sequence of the gonadotropin receptors. (*A*) The LHR and FSHR consist of a ~350-amino-acid-long extracellular domain (exodomain) and a membrane-associated domain (endodomain) of equal size. The exodomain is thought to include eight to nine Leu-rich repeats (LRRs), each consisting of a β strand (shown as arrow) and an α helix (shown as a column). A schematic model of the LRRs in the exodomain and the seven transmembrane domains in the endodomain is shown. The endodomain consists of three exoloops, three cytoloops, seven transmembrane domains, and the C-terminal tail. (*B*) The exodomain sequence of human FSHR is aligned based on the LRR motif with conserved residues in bold letters. (*C*) Likewise, the LRR sequences of human LHR are aligned.

In this review, the common and differential mechanisms of the LHR and FSHR will be described.

Exodomain and high-affinity hormone binding

There is ample evidence that the exodomain alone is capable of binding the hormone with high affinity. For example, the truncated exodomain lacking the endodomain binds the hormones with an affinity similar to or slightly better than wild-type affinity [7–9, 19]. When the exodomain was attached to the single TM of CD8, the hybrid protein was capable of binding the hormone with high affinity [20, 21]. The exodomain makes direct contacts with both subunits of hCG [22, 23]. The exodomain shows several discrete sites for hormone contacts [24] and peptides corresponding to three regions in the exodomain were capable of inhibiting hCG binding to the receptor [25]. In addition, a number of ionic amino acids in the exodomain is important for hormone binding [26] and some mutations in the exodomain abolished hormone binding [17, 27].

Leu rich repeats

There are several notable features in the structure of the exodomain. The sequence alignment shows a Leu-rich repeat (LRR) motif [28, 29] (fig. 1B, C). This motif is present in a large number of proteins that belong to the LRR family [30]. Each LRR consists of 20–28 amino acids and has a short β strand linked to an α helix approximately parallel to each other (fig. 1A). The LRR is stabilized by hydrophobic interactions among residues, particularly involving Leu and Ile. In the crystal structures of some LRR proteins, up to 15 LRRs form a nonglobular, horseshoe-shaped structure with an inner lining of curved parallel β strands and an outer lining of helices. The β strand inner lining interacts with the protein ligands [31, 32]. Based on the established LRR crystal structures, the glycoprotein hormone receptors have been modeled [33–36]. These computer models show a 1/3 donut structure consisting of seven to nine LRRs, and the inner lining presumably interacts with the hormones, the center of the LRRs, in particular, interacting with the central groove of the hormones [37]. There is evidence showing the importance of LRR1–6 in hormone binding [38].

Despite the general attention paid to and deluge of models and reports on the LRRs, little was known about whether LRRs are functional in the exodomains of gonadotropin receptors and whether all of the LRRs contact the hormones. Recently, the β-stranded Leu/Ile residues in all LRRs of the human FSHR and LHR were individually substituted with Ala (Ala-scan) and the resulting mutant receptors characterized [39]. The impact of the mutants on hormone binding affinity was variable as shown by the ratio of the wild-type Kd value divided by the mutant Kd values (fig. 2). Ratios less than 1 indicate hormone-binding affinities lower than the wild type, whereas those greater than 1 show binding affinities higher than the wild-type affinity. The results suggest that the Leu and Ile residues are important although in varying degrees. Since the conserved and important residues are spread around an LRR (fig. 1A), some of them are probably necessary for the structural integrity of LRRs. De-

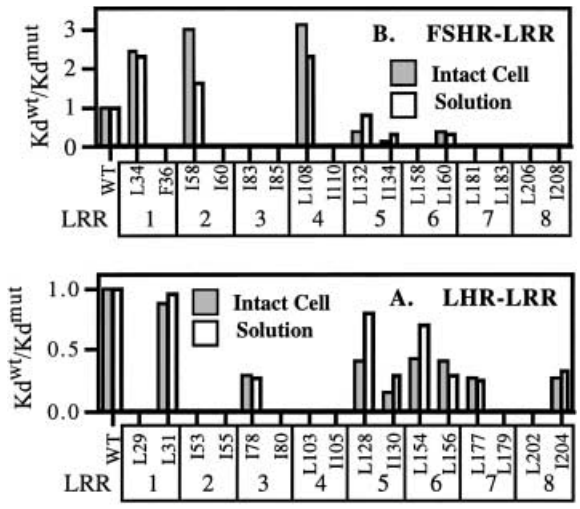

Figure 2. Comparison of LRR mutants. Leu and Ile residues in the putative β strands of LRRs of LHR and FSHR were individually substituted with Ala. The resulting mutant receptors were expressed and assayed for hormone binding, and the Kd values were determined. To readily compare these values, the wild-type Kd value was divided by each mutant Kd value. Resulting Kd ratios over 1 reflect affinities better than the wild type. Solid bars represent Kd values of receptors on intact cells and open bars, solubilized receptors.

spite the 94% homology in the Leu/Ile-X-Leu/Ile sequences between the two receptors, LHR and FSHR are capable of recognizing their cognate ligands, LH/CG and FSH, respectively, with high affinity and no cross-reactivity. Therefore, we need to know how the homologous Leu/Ile-X-Leu/Ile sequences in the putative hormone contact sites contribute to the discrete hormone specificity. The Kd ratios in figure 2 provide some answers, at least in part. For example, the location of the important LRRs is distinct. The upstream LRRs of LHR are more sensitive to Ala substitution than the downstream LRRs, whereas the downstream LRRs of FSHR are more sensitive (fig. 2). This suggests different contact points in LRRs of the two receptors. The role of LRRs may also differ, because Ala substitutions improved the binding affinity of some FSHR mutants, whereas Ala substitutions attenuated the binding affinity of LHR. This result suggests a clear difference in the structural role of LRRs in the two receptors, which could explain their dramatically distinct hormone-binding specificity. Despite the higher binding affinities of the FSHR mutants and the lower binding affinities of the LHR mutants, the EC_{50} values for cAMP induction increased for both receptors. Therefore, both receptors appear to share some common mechanism to generate the cAMP signal.

The 23 residues around LRR2 of LHR were also Ala-scanned. The results show that β-stranded Leu and Ile residues in all LRRs are important but not equally. These Leu/Ile-X-Leu/Ile motifs appear to form the hydrophobic core of the LRR loop, crucial for the LRR structure. Interestingly, the hot spots are primarily in the upstream and downstream LRRs of the LHR exodomain, whereas important LRRs are spread throughout the FSHR exodomain. This may explain their distinct hormone specificities despite the structural similarity, of the two receptors. Examination of the residues around the Ile-X-Ile sequence of LRR2 reveals the importance of the two alternate Ile residues, since the Ala substitutions for the intervening and flanking residues had less dramatic or marginal effects. In addition to the Ile-X-Ile sequence, Ala substitutions for Ile^{39}, Phe^{44}, Ile^{53}, Ile^{55} and Leu^{61}, which are conserved among LRRs, resulted in the complete loss of hormone binding without exception. These residues form the hydrophobic core of LRR2. In contrast, the substitution effects of other residues were less significant. These results support the LRR structure in LHR and its significant role in hormone binding.

Examination of LRR4 in LHR, in particular Leu^{103} and Ile^{105} in the putative β strand, shows that Leu^{103} and Ile^{105} are involved in the specific, hydrophobic interaction of the LRR4 loop, likely to form the hydrophobic core. This loop is crucial for the structural integrity of all LRRs. In contrast, the downstream sequence consisting of Asn^{107}, Thr^{108}, Gly^{109} and Ile^{110} of LRR4 is crucial for cAMP induction but not for hormone binding, folding and surface expression. This implicates its involvement in the interaction with the endodomain and signal generation. In particular, the N-terminal region of LRR4 assumes the hydrophobic core of the LRR4 loop, whereas the C-terminal region is crucial for signal generation. However, whether LRR4 interacts with hCG and the endodomain and how it might be involved in signal generation is unclear.

Disulfides and carbohydrates of the exodomain

The LHR exodomain has 12 Cys residues. Cys^{8}, Cys^{12}, Cys^{14} and Cys^{22} are located upstream of LRR1 and substitution for any of them abolishes receptor activity [17], consistent with our observation that the region interacts with hCG. Cys^{106} and Cys^{131} are in LRR4 and LRR5, respectively, and are not found in FSHR and thyroid-stimulating hormone receptor. They are important for hCG binding and are thought to form a disulfide bridge [17] and link LRR4 and LRR5 as suggested in computer models [34]. Therefore, they are considered important for hCG binding [17]. Cys^{253}, Cys^{254}, Cys^{317} and Cys^{327} are important for surface expression but not for hormone binding and receptor activation [17]. The LHR exodomain has six consensus N-glycosylation sites, which are actually glycosylated in transfected cells. After considerably controversy about their role, they are now generally agreed to be important for targeting to the cell surface but not for hormone binding and receptor activation [17].

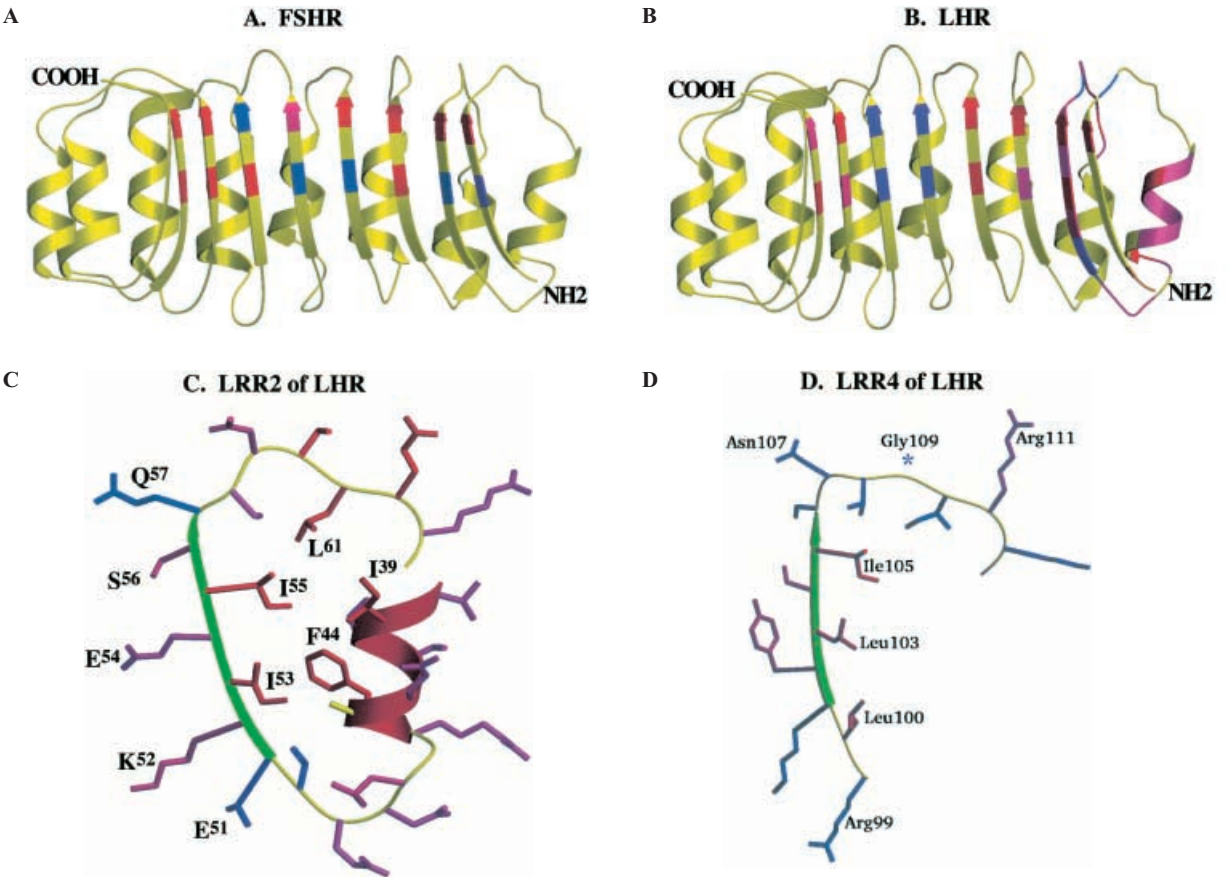

Figure 3. Exodomain models. FSHR (*A*), LHR (*B*), LRR2 of LHR (*C*) and LRR4 of LHR (*D*). The receptor exodomain was homology-modeled, as described previously [34], based on the coordinates of ribonuclease inhibitor from the Brookhaven data base as the template with the O and Frodo software packages [53] using an Indigo 2-Extreme (Silicon Graphics) and easv10 (Evans and Sutherland). They were modified based on the experimental data described in this review. Each LRR consists of a β strand and an α helix, which are linked by loops. Computer modeling suggests the eight LRRs of the LHR assume a 1/3 donut structure, in which the β strands of the LRRs form the inner lining. The inner lining is likely to play a role in hormone binding. In each case, yellow indicates an natural residue, red indicates Ala substitutions with a maximum effect on hormone binding (Kd of hormone binding to cells) and/or expression, blue indicates a minimum effect on hormone binding, and colors in between (red > crimson > magenta > blue) indicate the effects between these extremes. Note that in this scheme, the wild-type FSHR would be a crimson color. FSH apparently binds better to the mutant receptors, so these are going to be bluer than the wild type. LRR2 of LHR is shown with the green β strand and red α helix. LRR4 was modeled to form a hydrophobic core [39], similar to the hydrophobic cores of the LRRs found in the crystal structure of ribonuclease inhibitors [30]. Once the hydrophobic core is factored in with the side chains of Leu^{100}, Leu^{103}, Ile^{105} and Ile^{110}, the orientation of the side chains of other residues is determined by energy minimization.

Direct interaction of the N-terminal, LRR4 and hinge region of the LHR exodomain with the hCG α and β subunits

In addition to the LRRs, there are the N- and C-flanking regions of the LRRs in the exodomain, and their size is substantial. Interestingly, the N-flanking region of the LRRs makes strong contact with both subunits of hCG according to mutational analysis and affinity labeling [24, 40]. Ala substitutions of some amino acids in the Asp^{17}–Arg^{26} region upstream of LRR1 completely impairs hCG binding to the LHR [24]. To determine whether the region interacts with hCG, the hormone was photoaffinity labeled with a peptide mimic corresponding to Gly^{18}–Tyr^{36} of the receptor (LHR^{18-36}). This peptide was derivatized with a UV-activatable agent 4-azidobenzoyl (AB) or 4-azidobenzoylglycyl (ABG) at the amino terminus and radioiodinated to produce ^{125}I-AB-LHR^{18-36} or ^{125}I-ABG-LHR^{18-36}. Interestingly, hCGα was preferentially labeled, suggesting specificity [40]. The labeling is saturable, dependent on the UV irradiation time and the concentration of the peptide and hCG. The labeling requires the UV activatable group, UV irradiation, the peptide derivatives and hCG. On the other hand, denatured hCG was not labeled and the mutant analog of the peptide with Ala substitutions for Leu^{20}, Cys^{22} and Gly^{24} failed to label hCG, as expected from the mutational analysis. In addition, the labeling was blocked by unla-

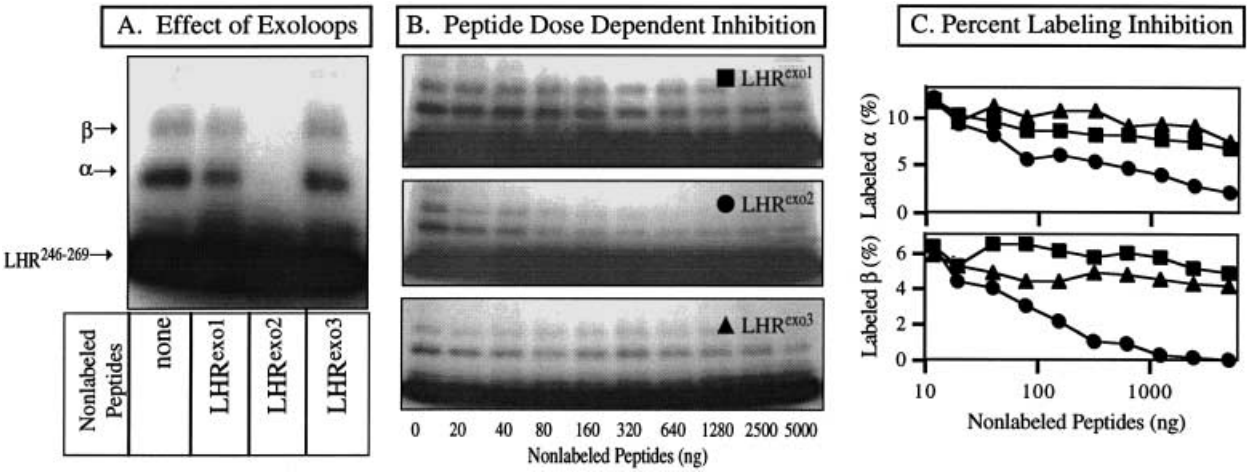

Figure 4. Effects of exoloop 1, 2 and 3 peptides on photoaffinity labeling of hCG by AB-^{125}I-LHR$^{246-269}$. hCG and AB-^{125}I-LHR$^{246-269}$ were incubated with 7000 ng of nonlabeled LHR exoloop 1, 2 or 3 peptides and exposed to UV (*A*). hCG and AB-^{125}I-LHR$^{246-269}$ were incubated with increasing concentrations of nonlabeled LHR exoloop 1, 2 or 3 peptides and exposed to UV (*B*). The samples were electrophoresed, and the resulting gels were autoradiographed. The graphs show the percent radioactivity of the α and the β band in a gel lane (*C*).

beled wild-type peptide but not by the mutant peptide. These results corroborate the mutational analysis showing the importance of the three residues in hormone binding. Further, they indicate the specificity of the photoaffinity labeling. The results indicate that the region upstream of LRR1 interacts with hCG and the contact points are in close proximity to both subunits of hCG. In particular, the alternate residues, Leu^{20}, Cys^{22} and Gly^{24}, are crucial for hCG binding and photoaffinity labeling. In addition, the results underscore the fact that there is a crucial hormone contact site outside the popularly believed primary hormone-binding sites, LRRs.

The affinity labeling of hCG with the synthetic peptide corresponding to LHR LRR4 shows that the N-terminal region of LRR4 interacts with hCG, preferentially the hCGα subunit, and that the hCG/LRR4 complex interacts with exoloop 2 of the endodomain [41]. This interaction offers a mechanism to generate hormone signal. Furthermore, the downstream flanking sequence (hinge region), Thr^{250}–Gln^{268}, of the LRRs specifically interacts with hCG, preferentially hCGα [42]. This interaction is inhibited by exoloop 2 of the endodomain but not by exoloops 1 and 3, suggesting an intimate relationship between Thr^{250}–Gln^{268}, exoloop 2 and hCG (fig. 4). Taken together, our observations suggest a new paradigm: that the LRRs contact the front of hCG while both flanking regions of the LRRs interact with the sides of hCG. This would trap hCG in the 1/3 donut structure of the LRRs and enhance the binding affinity. In addition, mutations of conserved Ser^{255} in the sequence can constitutively activate the receptor [42, 43]. This provides a clue for the enigmatic signal modulator in the exodomain.

Conformational adjustment upon hCG binding to the receptor

The earliest evidence for the conformational adjustment came from deglycosylated hCG which binds to the LH/CG receptor but fails to activate it [44]. The addition of antibodies to hCGβ to the deglycosylated hCG/receptor complex activated the receptor [45], suggesting that a structural change in hCGβ leads to receptor activation. Alternatively, the bulky antibodies bound to hCGβ might have caused a conformational change in the hormone/receptor complex. This latter possibility is, however, less likely, since antibodies to hCGα did not activate the receptor. To follow up the study and obtain more direct evidence, the interaction between the hCG$\alpha\beta$ subunits was examined using a photoactivatable derivative of hCG. Here, a free α subunit was derivatized with the photoactivatable reagent ABG, and reassociated with unmodified β subunit to produce ABG-α/β. ABG-α/β was capable of high-affinity receptor binding and activation [46]. ABG attached to amino groups of the α subunit was capable of cross-linking the α to the β subunit (fig. 5). However, the extent of inter-subunit cross-linking of ABG-^{125}I-α/β bound to the receptor was about three-fold less than unbound ABG-^{125}I-α/β [16]. This dramatic difference indicates structural change at the hCG subunit interface in response to hCG binding to the receptor. Furthermore, the change occurs before the secondary interaction between the hCG/exodomain complex and the endodomain [22]. Since the reagent reacts specifically with amino groups, one or more of the Lys residues of hCGα was derivatized with the reagent and involved in the $\alpha\beta$ dimer cross-linking. The results show that Lys residues appear to respond to hCG binding to the receptor and might be involved in

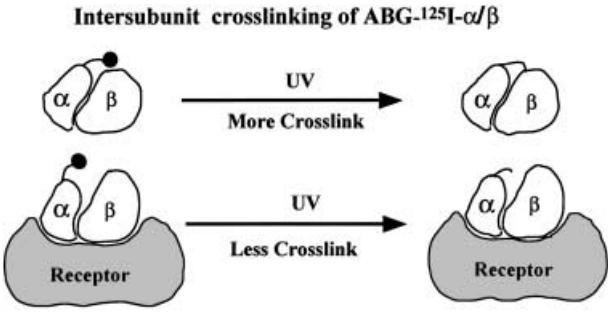

Figure 5. Hypothetical presentation of receptor-dependent intersubunit cross-linking of hCG. The intersubunit interaction of hCGα and β changes upon receptor binding. Therefore, the intersubunit cross-linking of the hCG dimer by attached photosensitive reagent changes. The change in the intersubunit interaction is exaggerated.

receptor activation. The α subunit has five Lys residues, including the penultimate Lys[91].

Endodomain, receptor activation and two distinct signals

Hormone binding activates the receptor, which is generally described as receptor activation. The seemingly one-step process, however, comprises a series of multiple steps including signal generation, signal propagation and signal transfer (fig. 6). Hormone binding leads to conformational changes of a hormone and the receptor initially at the hormone-receptor interface (signal generation). This conformational change in the receptor is thought to propagate through the TM to the cytoplasmic part of the receptor (signal propagation).

Hormones generally activate more than one signal pathway when they bind to their receptors. For example,

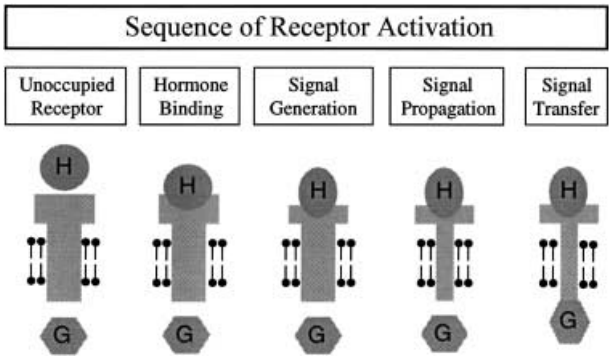

Figure 6. Steps of receptor activation. Receptor activation occurs in multiple steps after hormone (H) binding, which leads to a conformational change in the hormone receptor at the hormone-receptor interface (signal generation). The signal propagates through the TM region by conformational changes in the TM domain and impacts the structure of the cytoplasmic part of the receptor (signal propagation), which modulates the interaction with signal molecules such as G protein (G) (signal transfer).

LHR and FSHR activate two enzymes, adenylyl cyclase and phospholipase Cβ, and potentially, others [47]. When Lys[583] was substituted with a panel of amino acids, no substitutions for Lys[583] were permissible for activation of adenylyl cyclase despite successful surface expression and hormone binding. In contrast, several substitutions were permissible for phospholipase Cβ activation [18]. Lys[583] is located at the boundary of exoloop 3 and TM helix 7. Therefore, the amino acid at this position is expected to play a crucial role in maintaining the structure and orientation of TM7, thus influencing its function [14]. In fact, the substitution for Lys[583] with a variety of amino acids resulted in the loss of cAMP induction, regardless of side chain charge, length, shape and hydrophobicity, as did the deletion of Lys[583] [48]. These results suggest an irreplaceable and unique role and interaction of the Lys[583] side chain. In addition to the Lys at the boundary of exoloop 3 and TM7, the Asp between TM2 and exoloop 1 is also essential for the adenylyl cyclase/cAMP signal for both FSHR and LHR [49, 50]. TMs 2 and 7 are apposed in the membrane and, therefore, the Asp and Lys at the top of the two TMs are likely to be apposed and ion paired, which may play a crucial role in the signal generation of adenylyl cyclase.

In conclusion, adenylyl cyclase and phospholipase Cβ are activated by distinct signals from the receptor and the signals appear to originate from separate sites in the exoloops [18, 48]. In addition to the exoloops, the immediate upstream region of TM1 appears to be involved in activation of adenylyl cyclase [51]. Therefore, these extracellular regions are likely to contact the hCG/exodomain complex and play a role in signal generation.

Constraint of hormone binding to the exodomain by exoloops 2 and 3

Exoloops are logical candidates for interaction with the exodomain. LHR exoloop 3, connecting TMs 6 and 7, has been implicated in the signaling of the LHR [17, 52]. The 11 residues of exoloop 3 were Ala-scanned and the resulting mutants were assayed for hCG binding and cAMP induction. The Kd values, EC_{50} for cAMP induction and maximum cAMP level were determined [21]. To readily compare these values, the wild-type Kd value was divided with each mutant Kd value (table 1). Likewise, the wild-type EC_{50} for cAMP induction was divided with each individual mutant value. In addition, the maximum cAMP levels of individual mutants were divided with the wild-type maximum cAMP level. The resulting Kd and EC_{50} ratios over 1 reflect affinities better than the wild type. Similarly, a maximum cAMP level over 1 means that it is higher than the wild-type level. The $Kd^{wt/mut}$ ratios show that some Ala substitutions significantly im-

Table 1. Effects of Ala substitution for exoloop 3 amino acids.

	WT	K573	V574	P575	L576	I577	T578	V579	T580	N581	S582	K583
$Kd^{wt/mut}$	1.00	1.43	0.70	0.92	1.28	1.33	1.31	0.93	1.01	3.35	1.06	1.68
$EC_{50}^{wt/mut}$	1.00	0.56	0.34	0.12	0.51	0.25	0.80	0.37	0.61	0.43	0.83	none
Max $cAMP^{mut/wt}$	1.00	0.88	0.95	0.28	0.77	0.74	0.83	0.53	0.83	0.80	0.95	0

Exoloop 3 residues were individually substituted with Ala and the resulting mutant LHRs were individually expressed on 293 cells. hCG binding and cAMP induction were assayed. The Kd value and EC_{50} value of the wild type (WT) was divided with the corresponding values of the mutants.

proved the binding affinity, in particular the three-fold increase in the binding affinity of the Asn^{581}Ala mutant [48]. The result suggests the potential interaction of exoloop 3 with the exodomain. On the other hand, all of the $EC50^{wt/mut}$ ratios for cAMP production decreased, indicating that Ala substitutions impaired the affinity for cAMP induction. Some were severely impaired, and are positioned at one side of exoloop 3. These severely impaired residues could be involved in either interacting with the exodomain or generating the AC/cAMP signal, or both. Another notable feature is that the Lys^{583}Ala substitution resulted in the complete loss of cAMP induction, whereas the hormone-binding affinity improved by ~70%. These results suggest that the exodomain and endodomain interact and mutually modulate one another. Significantly, hormone binding and receptor activation are antagonistic in some cases. The receptors apparently have solved this inevitable and crucial problem by a compromise, adopting mutually acceptable levels of hormone binding and receptor activation.

LHR exoloop 2 constrains hormone binding to the exodomain. Exoloop 2 consists of 20 amino acids, Ser^{484}–Gln^{503}. Most of the mutants are expressed on 293 cells, bind hCG and induce cAMP in response to hCG (table 2), which is in contrast to exoloop 3 mutants. Ala substitution for several residues, Ser^{484}, Tyr^{486}, Cys^{492} and Pro^{494}, prevented surface expression. Interestingly, the affinity of hormone binding to intact cells or after solubilization for Ser^{484}, Asn^{485}, Lys^{488}, Ser^{490} and Ser^{499} is noticeably higher than the wild-type affinity. Computer modeling [21] suggests that these residues may be positioned on one side of exoloop 2 and constrain the hormone binding at the exodomain. To test this hypothesis, we carefully examined the binding affinity of the exodomain after truncating the endodomain. The truncation consistently improved the affinity by 30–100%. These results suggest the potential interaction of exoloop 2 with the exodomain. Exoloop 2 interacts with hCG, because it inhibits the affinity labeling of hCG with the LHR LRR4 and hinge peptides and, furthermore, it may interact with the hinge region and LRR4 of the exodomain.

Suppression and activation of signal generation in the endodomain by the exodomain

Since exoloops constrain the hormone binding to the exodomain, is it possible for the exodomain to affect the signal generation in the endodomain? This question is particularly relevant, because LRR4 and the hinge region of the exodomain appear to interact with exoloop 2. Ser^{255} in the hinge region is conserved in the LHR and FSHR among species and acts as a suppressor of the adenylyl cyclase signal. Similarly, Gly^{109} in LRR4 is conserved in the LHR and FSHR among species but appears to be an activator. Considering the interaction of the hinge region and LRR4 with exoloop 2, Ser^{255} and Gly^{109} play a pair of counteracting switches, a suppressor and an activator, and

Table 2. Effects of Ala substitution for exoloop 2 amino acids.

	WT	S484	N485	Y486	M487	K488	V489	S490	I491	C492	L493	P494
$Kd^{wt/mut}$	1.00	2.04	1.28	0.68	0.79	1.48	0.44	1.37	0.82	0.74	1.00	0.51
$EC_{50}^{wt/mut}$	1.00	–	0.25	–	0.34	0.23	1.03	0.30	0.19	–	0.24	–
Max $cAMP^{mut/wt}$	1.00	–	0.68	–	0.82	1.29	1.29	0.88	0.48	–	1.12	–

	M495	D496	V497	E498	S499	T500	L501	S502	Q503
$Kd^{wt/mut}$	0.44	1.03	0.82	0.82	1.32	0.93	1.03	0.59	0.90
$EC_{50}^{wt/mut}$	0.46	0.56	0.34	0.42	0.32	0.86	0.91	0.52	0.37
Max $cAMP^{mut/wt}$	0.78	1.22	0.85	1.08	0.82	1.12	1.08	0.92	0.97

Exoloop 2 residues were individually substituted with Ala and the resulting mutant LHRs were individually expressed on 293 cells. hCG binding and cAMP induction were assayed. The Kd value and EC_{50} value of the wild type (WT) was divided with the corresponding values of the mutants.

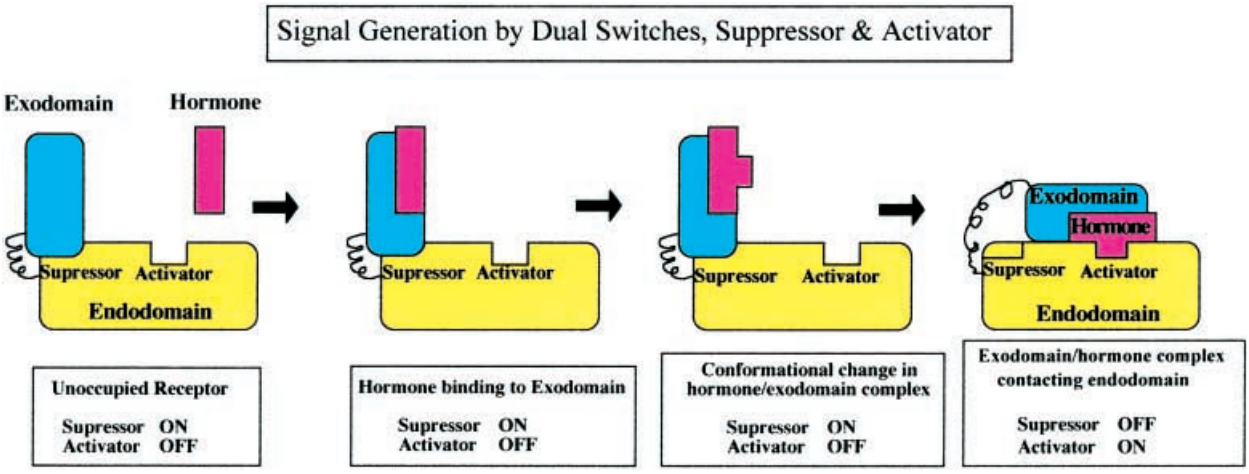

Figure 7. Signal suppressor and activator for receptor activation of the exodomain. There are a signal suppressor and activator in the exodomain, which undergo changes upon hormone binding and presumably interact with and modulate the endodomain for signal generation.

modulate the adenylyl cyclase signal, as suggested in figure 7. How these suppressor and activator interact with exoloop 2 and then control the signal generation, which involves exoloops 1 and 3, is unclear.

In conclusion, the exodomain and endodomain of the gonadotropin receptors are intimately associated before and after hormone binding. FSH, LH and hCG initially bind to the exodomain of their cognate receptors, and the resulting hormone/exodomain complex undergoes a conformational change [16] and modulates the endodomain. This secondary event generates distinct signals to activate adenylyl cyclase and phospholipase Cβ in the endodomain [14, 15, 17].

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