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A molecular dissection of the glycoprotein hormone receptors

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In glycoprotein hormone receptors, a subfamily of rhodopsin-like G protein-coupled receptors, the recognition and activation steps are carried out by separate domains of the proteins. Specificity of recognition of the hormones thyrotropin (TSH), lutropin (LH), human chorionic gonadotropin (hCG) and follitropin (FSH) involves leucine-rich repeats (LRRs) present in an N-terminal ectodomain, and can be associated with a limited number of residues at key positions of the LRRs. The mechanism by which binding of the hormones results in activation is proposed to involve switching of the ectodomain from a tethered inverse agonist to a full agonist of the serpentine, rhodopsin-like region of the receptor. Unexpectedly, the picture is complicated by the observation that promiscuous activation of one of the receptors (FSHr) by hCG or TSH can result from activating mutations affecting the serpentine region of the receptors.

The glycoprotein hormones and their receptors constitute an interesting example of co-evolution. The hormones follitropin (FSH), lutropin (LH), human chorionic gonadotropin (hCG) and thyrotropin (TSH) are dimeric proteins of ~30 kDa comprising a common α subunit and specific β subunits. The β subunits are encoded by paralogous genes that have substantial sequence similarity (Figure 1a). The corresponding receptors, FSHr, LHr or hCGr and TSHr, are members of the rhodopsin-like G protein-coupled receptor (GPCR) family. As such, they contain a 'serpentine' region containing seven transmembrane helices with many (but not all) of the sequence signatures typical of this receptor family. In addition – and a hallmark of the subfamily of glycoprotein hormone receptors (GpHRs) – they contain a large (350–400 residues) N-terminal ectodomain that is responsible for the high affinity and selective binding of the corresponding hormones [1–3] (Figure 2). The higher sequence identity of

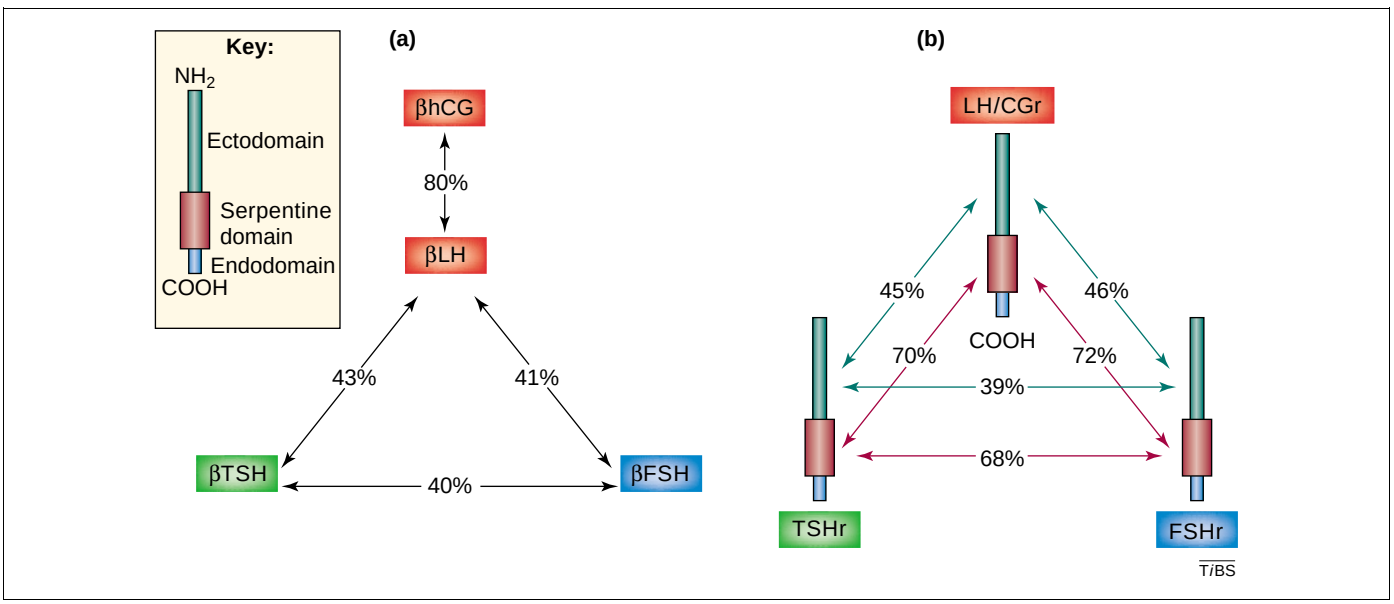

Figure 1. Both the β subunits of glycoprotein hormones **(a)** and the glycoprotein hormone receptors **(b)** are encoded by paralogous genes. Sequence identities are indicated (as percentages) for the β subunits of the four hormones (a) and separately for the ectodomains and serpentine domains of the three receptors (b). The pattern of shared similarities suggests co-evolution of the hormones and the ectodomain of their receptors, resulting in generation of specificity barriers. The high similarity displayed by the serpentine regions of the receptors is compatible with a conserved mechanism of intramolecular signal transduction. Abbreviations: FSH, follitropin; FSHr, FSH receptor; hCG, human chorionic gonadotropin; LH, lutropin; LH/CGr, (shared) LH and/or CG receptor; TSH, thyrotropin; TSHr, TSH receptor.

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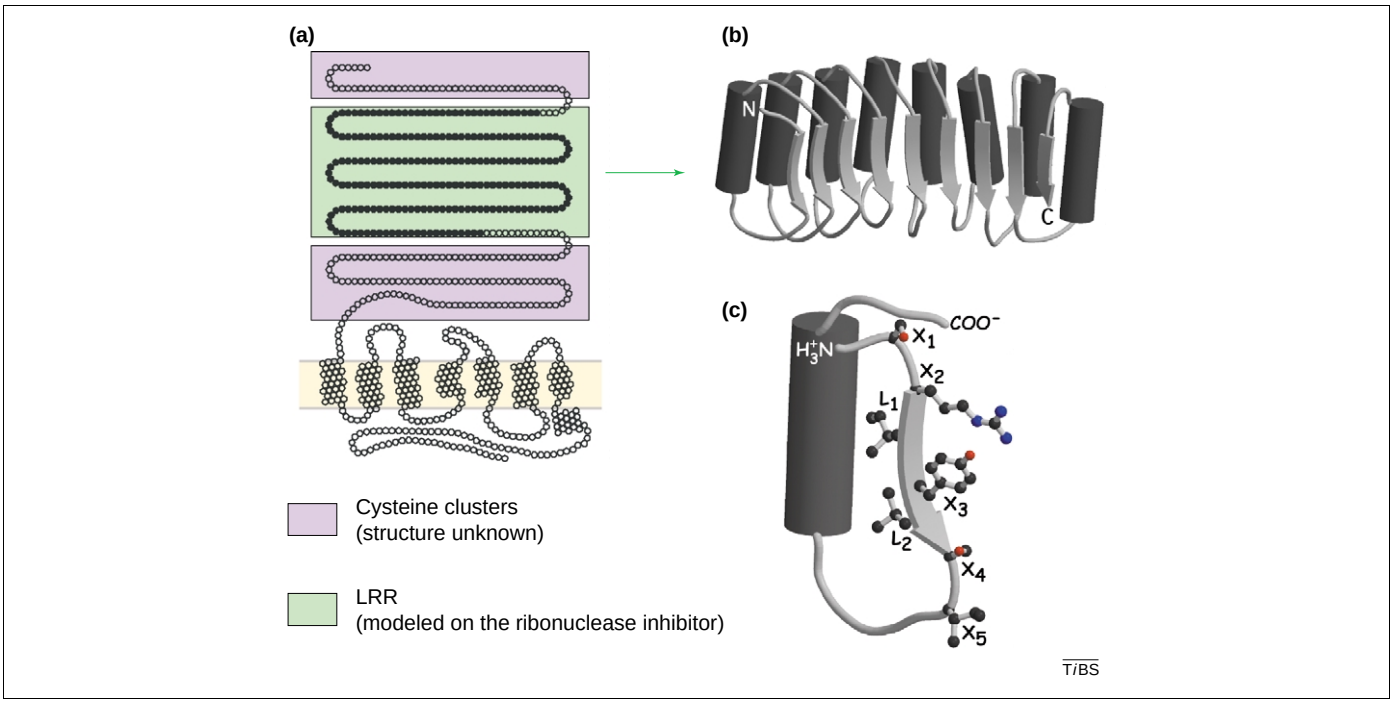

Figure 2. Schematic representation of the structures composing glycoprotein hormone receptors. **(a)** Two-dimensional representation with indication of the various domains. The purple boxes correspond to N-terminal and C-terminal cysteine-rich regions of the ectodomain, flanking nine leucine-rich repeats (LRR; green box). **(b)** Repeats are made of 20–24 amino acids forming a β strand followed by an α helix. In LRR-containing proteins, the repeat units are arranged with their β strands (arrows) and α helices (cylinders) parallel to a common axis and organized spatially to form a horseshoe-shaped molecule, with the β strands and α helices making the concave and convex surfaces of the horseshoe, respectively. **(c)** Representation of a single LRR unit. The inner surface of the horseshoe is composed of seven residues: $x_1x_2Lx_3Lx_4x_5$. The side chains of the leucine residues are pointing inside the hydrophobic core of the protein and are important for its stability. The side chains of the x (representing any amino acid) residues are predicted to be exposed to the solvent, making the surface available for interaction with the ligands [59]. Reprinted, with permission, from Ref. [16] (http://www.nature.com).

the serpentine regions (~70%) when compared with the ectodomains (~40%; Figure 1b) suggested early on that the former are interchangeable modules capable of activating the G proteins (mainly the α subunit of stimulating G protein ($G_{\alpha s}$)] after specific binding of the individual hormones to the latter [4,5]. Contrary to other rhodopsin-like GPCRs, binding of the hormones to their ectodmains can be observed with high affinity in the absence of the serpentine [6–8]. The intramolecular transduction of the signal between these two regions of the receptors raises an interesting mechanistic issue.

Another issue, of an evolutionary nature, relates to the shaping of hormone–receptor couples to cope with the emergence of hCG in primates [2]. Whereas in all mammals the circulating concentrations of TSH, LH and FSH are in proportion with their dissociation constant (Kds) for the corresponding receptors (in the low nanomolar range), in primates – particularly humans – hCG, which shares its receptor with LH (referred to as LH/CGr), can approach micromolar concentrations during the first trimester of pregnancy. This constitutes a challenge to the specificity of recognition regarding the TSH and FSH receptors, and is known to be responsible for some 'spill-over' in gestational trophoblastic disease, in which even higher plasma levels of hCG are observed than in normal pregnancy.

The recognition step

Three-dimensional structures are available for hCG and FSH [9–11], whereas for the ectodomains of the receptors, we are left with structural models covering only part of their structure (Figure 2a,b). These are based on the known 3D structure of proteins containing leucine-rich repeats (LRRs) [12–14]. LRRs are 20–25-residue protein motifs consisting of a β strand and an α helix that are connected by a turn (Figure 2c). When assembled sequentially in a protein, the LRRs determine a horseshoe-like structure with the β strands making a concave inner surface (Figure 2b). This has been shown to constitute the binding interface of the first LRR-containing protein that has been crystallized: the ribonuclease inhibitor [15]. Nine such motifs are found in the ectodomains of GpHRs. The model structure of the LRR regions of receptors predicts that non-leucine residues ($x_{1,2,3,4,5}$; Figure 2c) would be pointing outwards and be available for interaction with the hormones, which immediately implicates them in recognition specificity. Flanking the LRR region of the ectodomain are two cysteine-rich domains, the 3D structure of which is completely unknown.

Extensive amino acid substitutions by site-directed mutagenesis of the X residues in the LRR region of TSHr and FSHr (Figure 2c) for their counterparts in the LH/CGr provided strong support for this model [16]. Exchanging eight or two amino acids of the TSHr or FSHr, respectively, for the corresponding LH/CGr residues (Figure 3a), resulted in mutants displaying a sensitivity to hCG matching that of the wild-type LH/CGr [16]. Surprisingly, although gaining sensitivity to hCG, the mutants kept a normal sensitivity to their natural agonist, making them dual specificity receptors. For the TSH receptor, it is necessary to exchange twelve additional residues to fully transform it into a bona fide LH/CGr (T90; Figure 3c). From an

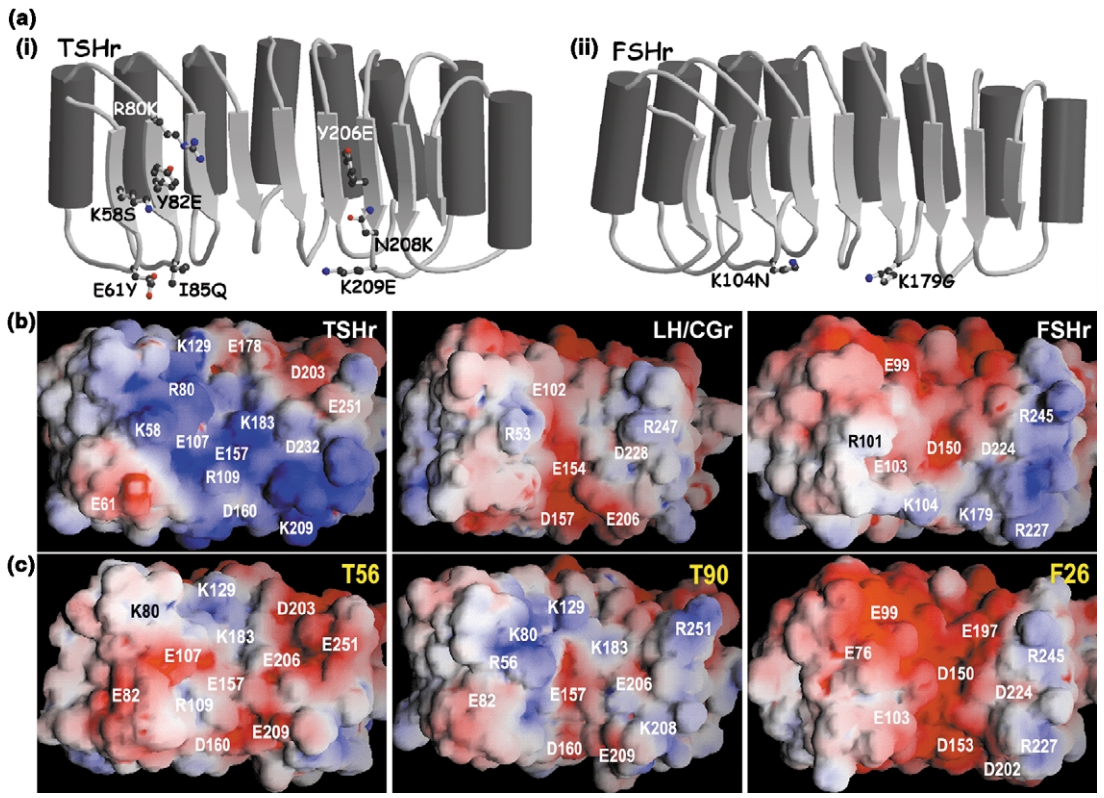

Figure 3. Exchange of recognition specificity of glycoprotein hormone receptors by substitution of selected residues in the β strands of the leucine-rich repeat (LRR) in their ectodomains. **(a)** Schematic representation of the LRR regions of (i) the thyrotropin receptor (TSHr) and (ii) the FSHr, with indication of the eight [T56 mutant; (i)] or two residues [F26 mutant; (ii)] leading to an increase in sensitivity to recombinant human chorionic gonadotropin (rhCG). **(b)** The molecular electrostatic potential at the accessible surface of the models established for TSHr and FSHr substitution mutants. Note, the important reduction of electropositivity of the surface of T56, especially at the lower part of the middle region of the horseshoe. This mutant displays sensitivity to rhCG similar to the lutropin (LH)/chorionic gonadotropin (CG) receptor (LH/CGr), while keeping nominal sensitivity to recombinant human TSH [16]. T90 mutant is a TSHr with twenty residues of the β strands of the LRR region exchanged with the LH/CGr. Its molecular electrostatic potential at the accessible surface closely reproduces the acidic groove of the wild-type LH/CGr. This mutant is as sensitive to rhCG as the LH/CGr and has completely lost sensitivity to recombinant human TSH [16]. The molecular electrostatic potential at the surface of the F26 mutant also displays an acidic groove. The main difference with the wild-type FSHr is the extension of the electronegative region to the lower-middle region of the horseshoe. The F26 mutant behaves functionally as a dual receptor, with increased sensitivity to rhCG and conserved sensitivity to rhFSH [16]. Adapted, with permission, from Ref. [16] (http://www.nature.com).

evolutionary point of view, these results indicate that nature has built recognition specificity of hormone–GpHR couples on both attractive and repulsive residues, and that residues at different homologous positions have been selected to this result in the different receptors.

Inspection of electrostatic surface maps of models of the three wild-type receptors and some of the mutants is revealing in this respect [16] (Figure 3b,c). The LH/CGr displays an acidic groove in the middle of its horseshoe, extending to the lower part of it (corresponding to the C-terminal ends of the β strands). Generation of a similar distribution of charges in the dual-specificity (T56) and reverse-specificity (T90) TSHr mutants suggests that this is important for hCG recognition. Transformation of the FSHr into a dual-specificity mutant (simply by mutating Lys104 and Lys179) is accompanied by a shift of positive to negative charges at the bottom part of its horseshoe (Figure 3b,c). This suggests that these two basic residues function as guards against promiscuous recognition of the wild-type FSHr by hCG [16]. Attempts to correlate charge distributions in the wild-type and mutant receptors with those of residues in the three hormones, suggests that the 'seatbelt' region of the β subunits (known to have a key role in recognition specificity [17–19]) might face the bottom border of the horseshoe.

As indicated, the extremely high plasma concentrations of hCG during the first trimester of pregnancy constitutes a challenge to the recognition specificity of GpHRs. This has been illustrated recently in two pathological situations resulting from spontaneous mutations in TSHr and FSHr. In TSHr, mutation of Lys183 to Arg (K183R) in the fifth LRR of the ectodmain causes illegitimate stimulation of the thyroid gland by hCG, which results in severe hyperthyroidism during pregnancy [20]. In the frame of our discussion, the consequences of this apparently mild amino acid substitution on the electrostatic surface map of the mutant receptor provided a structural rationale to the observed loss of specificity [21]. In the case of FSHr, two mutant FSH receptors were identified displaying promiscuous activation by hCG in patients with spontaneous ovarian hyperstimulation syndrome [22,23]. Surprisingly, in these cases, the mutant residues were located in the serpentine region of the receptor, outside the hormone-binding domain, indicating that functional specificity might be distinct from true recognition specificity.

In addition to the hormone-specific interactions genetically encoded in the primary structure of the ligands and the LRR region of the receptors, we have recently demonstrated the importance of non-hormone-specific ionic interactions involving sulfated tyrosines present in the

ectodomains of all three receptors [24] (Figure 4a). Similar to the interaction between von Willebrand factor and glycoprotein 1bα (Gp1bα) [25], sulfation of the three GpHRs contributes crucially to the binding affinity for the hormones without interfering with specificity. Whether the sulfated tyrosine residues interact directly with the hormones, or contribute to correct shaping of the functional ectodomains remains to be determined. A similar reasoning might apply to the role of sialylated or sulfated carbohydrate chains that are linked to the ectodomain of GpHRs. In this respect, it is worth noting that amino acid substitutions in the ectodomain of GpHRs could, in theory, alter carbohydrate structure and, hence, affect hormone binding.

Activation of the serpentine region of GpHRs

As they belong to the rhodopsin-like GPCR family and display many of the cognate signatures in primary structure, the serpentine regions of GpHRs are likely to share common mechanisms of activation with rhodopsin [26]. Crystallographic data are only available for the inactive conformation of rhodopsin [26]. Nevertheless, molecular scenarios that are based on a panel of experimental approaches involving site-directed mutagenesis, cross-linking and molecular modeling have been proposed for the activation phenomenon (for a recent review see Ref. [27]). Sequence signatures that are characteristic of the serpentine region of GpHR suggest, however, the existence of idiosyncrasies associated with their specific mechanisms of activation. In addition, over the past ten years, LH/CGr and, even more so, TSHr has been found to be activated by a wide spectrum of gain-of-function mutations [28–30]. In LH/CGr, such mutations cause pseudoprecocious puberty [30], a rare disease expressed only in males and transmitted on the autosomal dominant mode. In TSHr, in addition to germline mutations causing hereditary toxic thyroid hyperplasia [31], somatic mutations have been found to be responsible for the majority of autonomous thyroid adenomas [28] – a relatively frequent and easily diagnosed condition. Compilation of data available for both receptors identify >30 residues of which mutation causes constitutive activation. As many somatic mutations affecting a given residue have been found repeatedly in the TSHr (and do not involve hypermutable targets), it is likely that we are approaching a saturation map for spontaneous gain-of-function mutations. Attempts to translate this map into mechanisms of transition between inactive and active conformations of the receptors are underway, in the light of structural data for rhodopsin. Three sequence patterns affected by gain-of-function mutations deserve special mention and might help our understanding of GpHR activation.

Firstly, Asp6.44 (D633 in TSHr; D578 in LH/CGr) belongs to the 'Phe-Thr-Asp (FTD)' signature, a motif specific to GpHRs, at the cytoplasmic side of transmembrane helix VI (TM-VI; Figure 4a). When mutated to a variety of amino acids, including alanine, the result is constitutive activation in both TSHr and LH/CGr [28,32–34]. This suggested that the gain-of-function results from the breakage of one or more bonds, rather than the creation of a novel interaction(s) by the mutated residue. The observation, in a GpHR homolog of *Drosophila*, of a reciprocal mutation involving D6.44 of the FTD motif in TM-VI and Asn7.49 (N7.49) of the

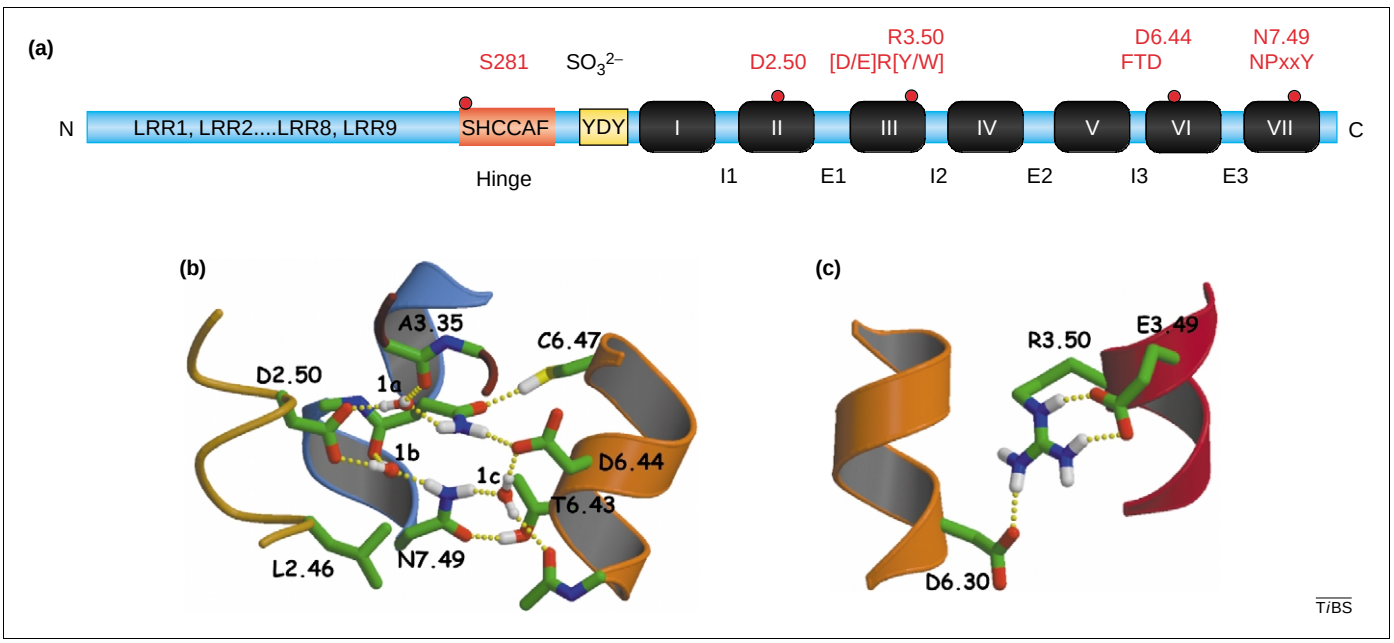

Figure 4. Sequence signatures common to all rhodopsin-like G protein-coupled receptors and sequence signatures specific to the glycoprotein hormone receptor (GpHR) gene family are both implicated in activation of GpHRs. **(a)** Linear representation of a typical GpHR, with indication of key residues (red circles) and conserved motifs: $SO3^{2-}$ stands for post-translational sulfation of the indicated tyrosine (Y) residues. The transmembrane helices are shown in black boxes; intracellular and extracellular loops are labeled I1–I3 and E1–E3, respectively. **(b)** Molecular modeling of the GpHR-specific lock between transmembrane helices VI and VII, involving mainly residues 6.43, 6.44 and 7.49. Rupture of this lock by mutation of Asp6.44 or Thr6.43 (D6.44 or T6.43, respectively) would release Asn7.49 (N7.49), making its side chain available for productive interactions (possibly with D2.50) and resulting in constitutive activation [32]. 1a, 1b and 1c represent water molecules, the position of which is modeled from the structure of rhodopsin [35]. **(c)** Molecular modeling of the ionic lock predicted to exist between the [D/E]R[Y/W] motif at the bottom of transmembrane helix III and an acidic residue in position 6.30 (D567 in the follitropin receptor). Rupture of this interaction also causes constitutive activation [22,60].

'Asn-Pro-Xaa-Xaa-Tyr (NPxxY)' motif in TM-VII (Figure 4a), suggests that an interaction between D6.44 and N7.49 would exist in the inactive conformation of GpHRs [32]. Figure 4b shows a molecular model in which a set of water molecules observed in the D2.50/N7.49 environment of Rhodopsin has been included [35] (indicated as 1a, 1b and 1c in Figure 4b). Constitutive activation would, thus, be the consequence of breakage of the interaction between Thr6.43 (T6.43) or D6.44 with N7.49 (Figure 4b). Interestingly, TSHr constructs bearing the substitution of Asn7.49 for Ala (N7.49A) can no longer be activated by TSH, despite normal expression and binding of the hormone [32]. We tentatively conclude that, in the inactive conformation of GpHRs, the side chain of N7.49 is normally 'sequestered' by both T6.43 and D6.44, and that the active conformation(s) require(s) establishment of novel interaction(s) of N7.49. N7.49 of the NPxxY motif is one of the most conserved residues in rhodopsin-like GPCRs. It has been suggested, on the basis of a reciprocal substitution with D2.50 in the gonadotrophin-releasing hormone (GnRH) receptor, that N7.49 would be implicated in the activation mechanism [36,37] by the creation of an interaction with D2.50. The experimental data on rhodopsin structure have recently supported this suggestion [38]. Our observations are compatible with this hypothesis; they suggest that, in GpHRs, evolution has selected a novel motif in TM-VI to control an activation switch common to all rhodopsin-like receptors. Whether this is related to the peculiarities of the activation mechanism of GpHRs involving their large ectodomain remains to be determined.

Secondly, Glu3.49 (E3.49) and Arg3.50 (R3.50) of the highly conserved '[Asp/Glu]-Arg-[Tyr/Trp] ([D/E]R[Y/W])' motif at the bottom of transmembrane III (TM-III) form an ionic lock with D6.30 at the cytoplasmic end of TM-VI (Figure 4a,c). Disruption of this ionic lock by either the mutation of E3.49 to Ala or Gln (E3.49A or E3.49Q, respectively) or mutations affecting D6.30 leads to constitutively active mutant receptors [39]. Thus, the movements of TM-III and TM-VI at the cytoplasmic side of the membrane is necessary for receptor activation [40].

Thirdly, Ser281 (S281) belongs to a GpHR-specific 'Tyr-Pro-Ser-His-Cys-Cys-Ala-Phe (YPSHCCAF)' sequence signature located downstream of the LRR region, in the ectodomain of the receptors (Figure 4a). After mutation of this serine residue had been shown to activate TSHr constitutively [41,42], this segment – sometimes referred to as the 'hinge' motif, was shown to play an important part in activation of all three GpHRs [43]. The functional effect of substitutions of S281 in the TSHr, or S277 in the LH/CGr, probably results from a local 'loss of structure' because the more de-structuring the substitutions, the stronger the activation [43,44]. This observation, together with results showing that mutation of specific residues in the extracellular loops of the TSHr cause constitutive activation [45], led to the hypothesis that activation of the receptor could result from the rupture of an inhibitory interaction between the ectodomain and the serpentine domain [41].

Finally, it is important to note that N7.49 of the NPxxY motif seems to be involved in stabilization of both the inactive and active states of the receptor. This is because the basal activity levels of all activating mutations, including those affecting the [D/E]R[Y/W] motif at the cytoplasmic side or S281 in the ectodomain, were significantly decreased by suppression of the sidechain of N7.49 (N7.49A double mutants) [39].

Interaction between the ectodomain and the serpentine domain

The hypothesis that the ectodomain would exert an inhibitory effect on an inherently noisy rhodopsin-like serpentine domain is supported by early data showing that mild treatment of TSHr-expressing cells with trypsin causes partial activation of the receptor [46]. Definite demonstration of such an effect was made by Zhang *et al.* [47], who showed that activation of the TSHr is secondary to 'beheading' in N-terminal truncated mutants. However, careful comparison of the activity of truncated mutants with maximally stimulated wild-type TSHr or S281 gain-of-function mutants, indicated that truncation of the receptor of its ectodomain resulted only in partial activation of the serpentine domain [48]. In addition, engineering activating mutations in the serpentine region of an N-terminally truncated mutant results in further activation of the constructs [48]. Interestingly, only mutations in the transmembrane helices are effective; substitutions in the extracellular loops of serpentine-only constructs were without effect [48].

From these observations, we proposed the following model for activation of the TSHr [48] (Figure 5a). In the resting state, the ectodomain would exert an inhibitory effect on the activity of an inherently noisy rhodopsin-like serpentine, qualifying pharmacologically as an inverse agonist of the serpentine. Upon activation, by binding of the hormone, or secondary to mutation of S281 in the hinge region, the ectodomain would switch from inverse agonist to full agonist of the serpentine region. The ability of the strongest S281 mutants to activate the receptor fully in the absence of hormone suggests that the ultimate agonist of the serpentine domain would be the 'activated' ectodomain, with no need for an interaction between the hormone and serpentine domain. The ineffectiveness of mutations in the extracellular loops to activate serpentine-only constructs suggests that, in the wild-type receptor, the exoloops and a region of the ectodomain (possibly the hinge region) cooperate in the generation of a structural module functioning as an agonist of the serpentine. Results obtained with chimeras between the FSHr and leucine-rich-repeat-containing G protein-coupled receptors (LGRs) agree with this model and suggest that the second exoloop has a key role in this mechanism [49]. Notably, direct interaction of the hormone with the serpentine region is not required in this model, but it is by no means excluded.

Unexpected complications

According to an appealing evolutionary scenario, and fitting with the functional data analyzed, GpHRs would have evolved from two distinct genes: the first encoding a typical rhodopsin-like serpentine receptor, the second encoding multiple LRR domains involved in protein–protein

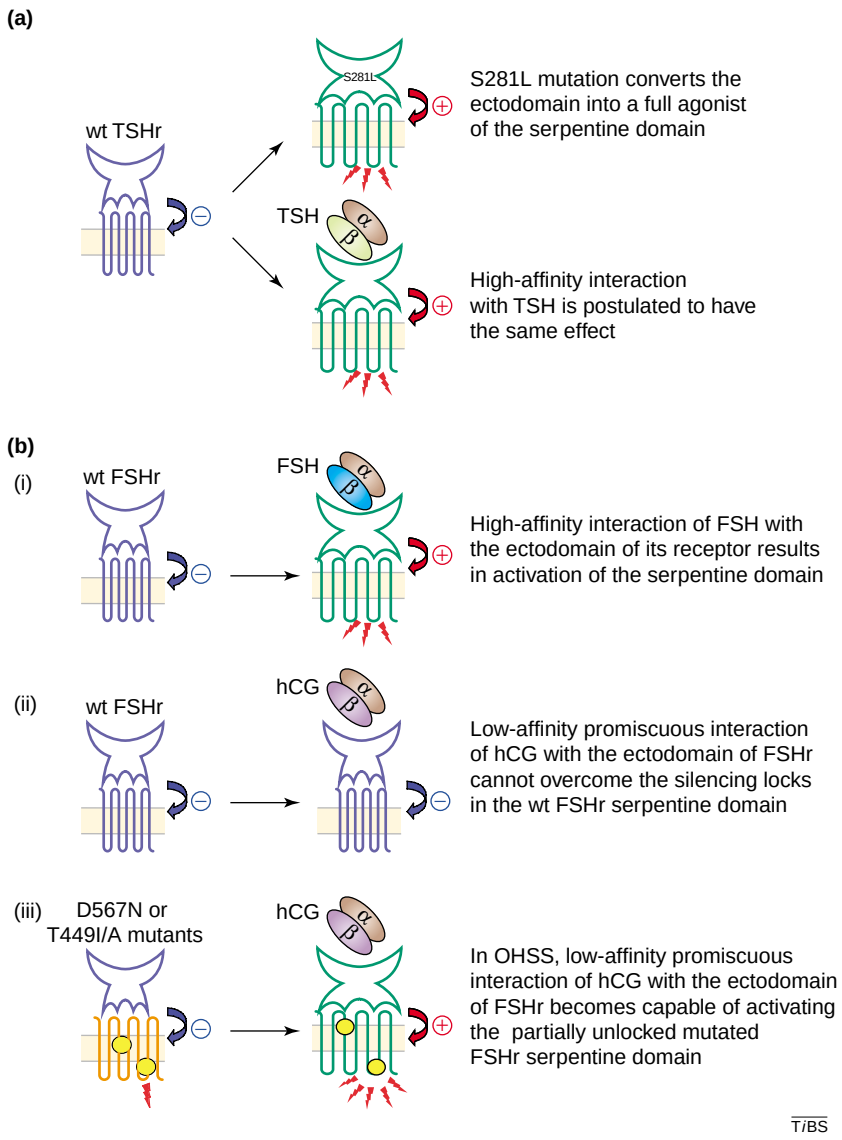

Figure 5. Interactions between the ectodomain and the serpentine domains are implicated in the activation mechanism and functional specificity. The receptors are represented with their ectodomain containing a concave, hormone-binding structure facing upwards, and a transmembrane serpentine region. **(a)** Our current model for activation of the wild-type (wt) thyrotropin receptor (TSHr). The basal state of the receptor is characterized by an inhibitory interaction (−) between the ectodomain and the serpentine domain. The ectodomain would function as a tethered inverse agonist of the serpentine region. Mutation of Ser281 in the ectodomain to leucine (S281L) switches the ectodomain from an inverse agonist into a full agonist (+) of the serpentine domain. Binding of thyrotropin (TSH; indicated by αβ dimeric structure) to the ectodomain is proposed to have a similar effect, converting it into a full agonist of the serpentine region. The serpentine regions in the basal (silent) state are represented as compact, blue structures. Fully activated serpentine regions are depicted as relaxed green structures, with red sparks indicating activation of the α subunit of stimulating G protein ($G_{\alpha s}$). **(b)** Our current interpretation of the phenotypes displayed by the substitution of Asp567 for Asn (D567N) or Thr449 for Ile (T449I) follitropin receptor (FSHr) mutants of patients with spontaneous ovarian hyperstimulation syndrome (OHSS) [22,23], in light of the model in (a). High-affinity interactions of the ectodomain of the wt FSHr with follitropin (FSH) results in full activation of the serpentine region (bi). High concentrations of human chorionic gonadotropin (hCG), although capable of establishing low-affinity interactions with the ectodomain of the FSHr, would be ineffective in activating a strongly locked wt serpentine domain (bii). In both D567N and T449I OHSS mutants (location of the mutations indicated by yellow circles), partial activation of the serpentine domains (indicated as a partially relaxed structure in orange) would lower the activation threshold of the serpentine, thus, enabling stimulation (biii). Adapted, with permission, from Ref. [48]. © (2002) The Endocrine Society.

interactions [50]. The ancestral gene encoding GpHR must be extremely old because a similar genomic organization is found in LGRs, members of which are already present in sea anemones [51]. All this points to a neat dichotomy between hormone recognition (by the LRRs in the ectodomain) and activation of the G protein (by the serpentine domain). This division of labor fits well with the data reported here. It is also supported by the identification of natural and experimental mutations in the ectodomain of GpHRs that affect specificity of hormone recognition [16,20]. Recent data, however, challenge this view, and make it necessary to revise the tight functional dichotomy between recognition and activation.

It is revealing that, again, the way was paved by natural mutations identified in patients with an interesting phenotype. Two families were identified in which female patients presented with spontaneous ovarian hyperstimulation syndrome (spontaneous OHSS) and mutation in their FSHr [22,23]. In the majority of cases, this condition is caused by excessive stimulation of the ovaries by exogenous gonadotropins administered in the context of *in vitro* fertilization procedures (iatrogenic OHSS) [52]. In the two

families studied, the disease occurred spontaneously on the occasion of each pregnancy. In both cases, it was shown that the mutated FSH receptors were abnormally sensitive to the pregnancy hormone hCG, thus providing a satisfactory explanation to the phenotype. The surprise was that the amino acid substitutions were located in the serpentine region of the FSHr, rather than the LRR region as would be expected [Figure 5b(iii)]. One of the mutations, D567N [22], affects residue 6.30 (previously discussed; Figure 4c). The phenotype of the mutant receptors was studied in more detail by additional site-directed mutagenesis and functional assays in COS cells (fibroblasts from African green monkey kidney), and the following picture emerges [22]: (i) in addition to enabling promiscuous stimulation by hCG, both mutations cause increased constitutive activity of a normally silent FSHr; (ii) the loss of specificity is not restricted to hCG, the mutants also show increased sensitivity to TSH; and (iii) other unrelated amino acid substitutions that cause increased constitutive activity of the FSHr display the same phenotype in terms of loss of specificity towards hCG and TSH.

These observations imply that low-affinity interactions between the ectodomain of the FSHr and hCG do occur normally during pregnancy, but are ineffective on the wild-type FSHr [Figure 5b(ii)]. They suggest the existence of a barrier to stimulation of the serpentine domain of FSHr by its activated ectodomain (the immediate agonist). In agreement with this view, the FSHr is reportedly more resistant to activation by gain-of-function mutations than the two other GpHRs [53]. In FSHr, activating mutations in the serpentine region, as in the two families with OHSS, would lower the barrier of activation of the serpentine region, thus enabling stimulation by low-affinity hCG (or TSH)–ectodomain complexes (Figure 5b).

Concluding remarks and future perspectives

It is remarkable that the study of spontaneous gain-of-function mutations has played a major part in our understanding of the structure–function relationships of the GpHRs, while simultaneously providing novel insight into the pathophysiology of a series of endocrine diseases. We believe, however, that we are close of having reached the limits of this approach and of its extension, site-directed mutagenesis. The challenge is now to acquire direct structural data and develop new strategies to explore the conformational changes in the hinge region of GpHRs, which will be central to our understanding of the activation mechanisms. This will probably require direct probing of structural changes by physicochemical means. The recent generation of monoclonal antibodies with potent TSH-stimulating activity provides new tools that might be of great help in such studies [54–56]. Finally, recent data indicate the ability of GpHRs to di(multi)merize [57,58]; further work will be required to include these observations in our current models.

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References

1 Dias, J.A. and Van Roey, P. (2001) Structural biology of human follitropin and its receptor. *Arch. Med. Res.* 32, 510–519
2 Szkudlinski, M.W. *et al.* (2002) Thyroid-stimulating hormone and thyroid-stimulating hormone receptor structure-function relationships. *Physiol. Rev.* 82, 473–502
3 Ascoli, M. *et al.* (2002) The lutropin/choriogonadotropin receptor, a 2002 perspective. *Endocr. Rev.* 23, 141–174
4 Braun, T. *et al.* (1991) Amino-terminal leucine-rich repeats in gonadotropin receptors determine hormone selectivity. *EMBO J.* 10, 1885–1890
5 Nagayama, Y. *et al.* (1990) Extracellular domain chimeras of the TSH and LHCG receptors reveal the mid-region (amino acids 171–260) to play a vital role in high affinity TSH binding. *Biochem. Biophys. Res. Commun.* 173, 1150–1156
6 Schmidt, A. *et al.* (2001) Hormone-induced conformational change of the purified soluble hormone binding domain of follitropin receptor complexed with single chain follitropin. *J. Biol. Chem.* 276, 23373–23381
7 Remy, J.J. *et al.* (2001) Purification and structural analysis of a soluble human chorionogonadotropin hormone–receptor complex. *J. Biol. Chem.* 276, 1681–1687
8 Cornelis, S. *et al.* (2001) Purification and characterization of a soluble bioactive amino-terminal extracellular domain of the human thyrotropin receptor. *Biochemistry* 40, 9860–9869
9 Lapthorn, A.J. *et al.* (1994) Crystal structure of human chorionic gonadotropin. *Nature* 369, 455–461
10 Wu, H. *et al.* (1994) Structure of human chorionic gonadotropin at 2.6 Å resolution from MAD analysis of the selenomethionyl protein. *Structure* 2, 545–558
11 Fox, K.M. *et al.* (2001) Three-dimensional structure of human follicle-stimulating hormone. *Mol. Endocrinol.* 15, 378–389
12 Kajava, A.V. *et al.* (1995) Modeling of the three-dimensional structure of proteins with the typical leucine-rich repeats. *Structure* 3, 867–877
13 Jiang, X. *et al.* (1995) Structural predictions for the ligand-binding region of glycoprotein hormone receptors and the nature of hormone–receptor interactions. *Structure* 3, 1341–1353
14 Bhowmick, N. *et al.* (1996) Determination of residues important in hormone binding to the extracellular domain of the luteinizing hormone/chorionic gonadotropin receptor by site-directed mutagenesis and modeling. *Mol. Endocrinol.* 10, 1147–1159
15 Kobe, B. and Deisenhofer, J. (1993) Crystal structure of porcine ribonuclease inhibitor, a protein with leucine-rich repeats. *Nature* 366, 751–756
16 Smits, G. *et al.* (2003) Glycoprotein hormone receptors: determinants in leucine-rich repeats responsible for ligand specificity. *EMBO J.* 22, 2692–2703
17 Grossmann, M. *et al.* (1997) Novel insights into the molecular mechanisms of human thyrotropin action: structural, physiological, and therapeutic implications for the glycoprotein hormone family. *Endocr. Rev.* 18, 476–501
18 Moyle, W.R. *et al.* (1994) Co-evolution of ligand-receptor pairs. *Nature* 368, 251–255
19 Dias, J.A. *et al.* (1994) Receptor binding and functional properties of chimeric human follitropin prepared by an exchange between a small hydrophilic intercysteine loop of human follitropin and human lutropin. *J. Biol. Chem.* 269, 25289–25294
20 Rodien, P. *et al.* (1998) Familial gestational hyperthyroidism caused by a mutant thyrotropin receptor hypersensitive to human chorionic gonadotropin. *N. Engl. J. Med.* 339, 1823–1826
21 Smits, G. *et al.* (2002) Lysine 183 and glutamic acid 157 of the thyrotropin receptor: two interacting residues with a key role in determining specificity towards TSH and hCG. *Mol. Endocrinol.* 1, 722–735
22 Smits, G. *et al.* (2003) Ovarian hyperstimulation syndrome due to a mutation in the follicle-stimulating hormone receptor. *N. Engl. J. Med.* 349, 760–766
23 Vasseur, C. *et al.* (2003) A chorionic gonadotropin-sensitive mutation in the follicle-stimulating hormone receptor as a cause of familial

gestational spontaneous ovarian hyperstimulation syndrome. *N. Engl. J. Med.* 349, 753–759
24 Costagliola, S. *et al.* (2002) Tyrosine sulfation is required for agonist recognition by glycoprotein hormone receptors. *EMBO J.* 21, 504–513
25 Dong, J. *et al.* (2001) Tyrosine sulfation of glycoprotein I(b)α. Role of electrostatic interactions in von Willebrand factor binding. *J. Biol. Chem.* 276, 16690–16694
26 Palczewski, K. *et al.* (2000) Crystal structure of rhodopsin: A G protein-coupled receptor. *Science* 289, 739–745
27 Ridge, K.D. *et al.* (2003) Phototransduction: crystal clear. *Trends Biochem. Sci.* 28, 479–487
28 Parma, J. *et al.* (1997) Diversity and prevalence of somatic mutations in the TSH receptor and Gsα genes as a cause of toxic thyroid adenomas. *J. Clin. Endocrinol. Metab.* 82, 2695–2701
29 Refetoff, S. *et al.* (2001) Thyroid disorders. In *The Metabolic and Molecular Bases of Inherited Diseases* (Scriver, C.R. and Sly, W.S., eds), pp. 4029–4076, McGraw-Hill
30 Shenker, A. (2002) Activating mutations of the lutropin choriogonadotropin receptor in precocious puberty. *Receptors Channels* 8, 3–18
31 Duprez, L. *et al.* (1994) Germline mutations in the thyrotropin receptor gene cause non autoimmune autosomal dominant hyperthyroidism. *Nat. Genet.* 7, 396–401
32 Govaerts, C. *et al.* (2001) A conserved ASN in TM7 is a on/off switch in the activation of the TSH receptor. *J. Biol. Chem.* 276, 22991–22999
33 Lin, Z. *et al.* (1997) A model of the lutropin/choriogonadotropin receptor: insights into the structural and functional effects of constitutively activating mutations. *Protein Eng.* 10, 501–510
34 Neumann, S. *et al.* (2001) A free carboxylate oxygen in the side chain of position 674 in transmembrane domain 7 is necessary for TSH receptor activation. *Mol. Endocrinol.* 15, 1294–1305
35 Okada, T. *et al.* (2002) Functional role of internal water molecules in rhodopsin revealed by X-ray crystallography. *Proc. Natl. Acad. Sci. U. S. A.* 99, 5982–5987
36 Zhou, W. *et al.* (1994) A reciprocal mutation supports helix 2 and helix 7 proximity in the gonadotropin-releasing hormone receptor. *Mol. Pharmacol.* 45, 165–170
37 Sealfon, S.C. *et al.* (1995) Related contribution of specific helix 2 and 7 residues to conformational activation of the serotonin 5-HT2A receptor. *J. Biol. Chem.* 270, 16683–16688
38 Fritze, O. *et al.* (2003) Role of the conserved NPxxY(x)5,6F motif in the rhodopsin ground state and during activation. *Proc. Natl. Acad. Sci. U. S. A.* 100, 2290–2295
39 Claeysen, S. *et al.* (2002) A conserved Asn in TM7 of the TSH receptor is a common requirement for activation by both mutations and its natural agonist. *FEBS Lett.* 517, 195–200
40 Ballesteros, J.A. *et al.* (2001) Activation of the β2-adrenergic receptor involves disruption of an ionic lock between the cytoplasmic ends of transmembrane segments 3 and 6. *J. Biol. Chem.* 276, 29171–29177
41 Duprez, L. *et al.* (1997) Constitutive activation of the TSH receptor by spontaneous mutations affecting the N-terminal extracellular domain. *FEBS Lett.* 409, 469–474
42 Kopp, P. *et al.* (1997) Congenital hyperthyroidism caused by a solitary toxic adenoma harboring a novel somatic mutation (serine281(isoleucine) in the extracellular domain of the thyrotropin receptor. *J. Clin. Invest.* 100, 1634–1639
43 Nakabayashi, K. *et al.* (2000) Activation of the luteinizing hormone receptor following substitution of Ser-277 with selective hydrophobic residues in the ectodomain hinge region. *J. Biol. Chem.* 275, 30264–30271
44 Ho, S.C. *et al.* (2001) Effects of mutations involving the highly conserved S281HCC motif in the extracellular domain of the thyrotropin (TSH) receptor on TSH binding and constitutive activity. *Endocrinology* 142, 2760–2767
45 Parma, J. *et al.* (1995) Somatic mutations causing constitutive activity of the TSH receptor are the major cause of hyperfunctional thyroid adenomas: identification of additional mutations activating both the cAMP and inisitolphosphate-Ca^{++} cascades. *Mol. Endocrinol.* 9, 725–733
46 Van Sande, J. *et al.* (1996) Specific activation of the thyrotropin receptor by trypsin. *Mol. Cell. Endocrinol.* 119, 161–168
47 Zhang, M. *et al.* (2000) The extracellular domain suppresses constitutive activity of the transmembrane domain of the human TSH receptor: implications for hormone–receptor interaction and antagonist design. *Endocrinology* 141, 3514–3517
48 Vlaeminck, V. *et al.* (2002) Activation of the cAMP pathway by the TSH receptor involves switching of the ectodomain from a tethered inverse agonist to an agonist. *Mol. Endocrinol.* 16, 736–746
49 Nishi, S. *et al.* (2002) The ectodomain of the luteinizing hormone receptor interacts with exoloop 2 to constrain the transmembrane region: studies using chimeric human and fly receptors. *J. Biol. Chem.* 277, 3958–3964
50 Gross, B. *et al.* (1991) Composite structure of the human thyrotropin receptor gene. *Biochem. Biophys. Res. Commun.* 177, 679–687
51 Nothacker, H.P. and Grimmelikhuijzen, C.J. (1993) Molecular cloning of a novel, putative G protein-coupled receptor from sea anemones structurally related to members of the FSH, TSH, LH/CG receptor family from mammals. *Biochem. Biophys. Res. Commun.* 197, 1062–1069
52 Delvigne, A. and Rozenberg, S. (2002) Epidemiology and prevention of ovarian hyperstimulation syndrome (OHSS): a review. *Hum. Reprod. Update* 8, 559–577
53 Kudo, M. *et al.* (1996) 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.* 271, 22470–22478
54 Ando, T. *et al.* (2002) A monoclonal thyroid-stimulating antibody. *J. Clin. Invest.* 110, 1667–1674
55 Sanders, J. *et al.* (2002) Thyroid stimulating monoclonal antibodies. *Thyroid* 12, 1043–1050
56 Costagliola, S. *et al.* (2002) Generation of a mouse monoclonal TSH receptor antibody with stimulating activity. *Biochem. Biophys. Res. Commun.* 299, 891–896
57 Ji, I. *et al.* (2002) *Cis*- and *trans*-activation of hormone receptors: the LH receptor. *Mol. Endocrinol.* 16, 1299–1308
58 Lee, C. *et al.* (2002) Two defective heterozygous luteinizing hormone receptors can rescue hormone action. *J. Biol. Chem.* 277, 15795–15800
59 Kobe, B. and Kajava, A.V. (2001) The leucine-rich repeat as a protein recognition motif. *Curr. Opin. Struct. Biol.* 11, 725–732
60 Parma, J. *et al.* (1993) Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. *Nature* 365, 649–651