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Helix 3-Helix 5 interactions in Steroid Hormone Receptor Function

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Abstract

Steroid hormones working through their receptors regulate a wide variety of physiologic processes necessary for normal homeostasis. Recent years have witnessed great advances in our understanding of how these hormones interact with their receptors, and have brought us closer to the era of directed drug design. We previously described a novel intramolecular interaction between helix 3 and helix 5 which is responsible for a Mendelian form of human hypertension. Further studies revealed that this interaction is highly conserved throughout the steroid hormone receptor family and functions as a key regulator of steroid hormone receptor sensitivity and specificity. Here, we review the contribution of helix 3-helix 5 interaction to steroid hormone receptor activity, with an eye towards how this knowledge may aid in the creation of novel therapeutic agonists and antagonists.

Steroid hormones play a critical regulatory role in a wide variety of physiologic processes, including development, cellular differentiation, and maintenance of cellular homeostasis and blood pressure. Their effects are primarily achieved through activation of specific steroid hormone receptors (SHRs), a subgroup of the larger family of nuclear receptors (NRs), which are activated by specific interactions between the hormone agonist and a C-terminal ligand-binding domain (LBD). In recent years, the availability of high resolution three-dimensional structural information for the LBD of all five steroid hormone receptors has provided a detailed mechanistic understanding of steroid receptor agonism and antagonism [1–8]. Insights gained from such study have greatly improved our understanding of the molecular requirements for ligand-binding and receptor activation and inhibition, and this will prove invaluable as we move forward into the era of directed drug design. However, the relationship between ligand-binding and receptor activation remains poorly understood, and events required beyond ligand-binding for receptor activation have not been well-defined.

The LBDs of NR's share a common architecture, with 12 alpha helices and one beta turn arranged around a central hydrophobic core [9]. Comparisons of the crystal structures of ligand-free and ligand bound NRs have shown that, upon ligand binding, NRs undergo a conformational change, including movement of helix 12 (H12) and a bending of helix 3 (H3) towards helix 5 (H5). The repositioned H12 aligns itself with H3 and H5 to form a pocket where transcriptional coactivators bind [9,10]. In recent years, the precise ligand-receptor contacts which allow these molecular events to occur have begun to be elucidated. Residues within the LBDs of nuclear receptors which interact with specific steroid functional groups have been identified, providing a structural basis for the steroid specificity of these receptors [11–17]. For example, in the human mineralocorticoid receptor (hMR), conserved residues in H3 and H5, Q776 and R817, form hydrogen bonds with the 3-ketone group of steroid hormones

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to anchor aldosterone's A-ring, while conserved residues in H3 and H12, N770 and T945, mediate binding of the D-ring [5,11]. The mineralocorticoid specificity of MR is thought to be mediated, at least in part, by a hydrogen bond between N770 on H3 and the C21-OH group of mineralocorticoids [11]. Such ligand-receptor interactions have now been described for a number of different steroid/nuclear hormone receptors [18].

A gain-of-function mutation in the mineralocorticoid receptor

We became interested in the regulation of SHR activity in trying to understand the mechanism by which a mutation in the mineralocorticoid receptor (MR) causes severe hypertension exacerbated by pregnancy in humans [19]. The mutation, a substitution of leucine for serine at residue 810 lies in the LBD of the receptor [19], and so we questioned whether it might alter the specificity or sensitivity of the receptor. We therefore studied the activity of the mutant receptor in *in vitro* transient transfection assays. While the mutation did not significantly alter the response of the receptor to mineralocorticoids such as aldosterone, we found that the mutation alters the specificity of the receptor, allowing mineralocorticoid antagonists such as spironolactone and progesterone to function as agonists [19]. Consistent with this, we found that female MR_{S810L} carriers had extraordinary elevations of blood pressure during pregnancy [19]. More recently, it has been determined that cortisone, a normally inactive byproduct of cortisol metabolism by 11 β -hydroxysteroid dehydrogenase type 2 in the distal nephron, is a potent agonist of the mutant receptor, thereby explaining the presence of hypertension in male and nonpregnant female MR_{S810L} carriers [19].

The finding that a single amino acid substitution in the hormone-binding domain of the receptor could so markedly alter receptor specificity led us to wonder how this change occurred. To determine the structural basis for the altered specificity, we created an atomic model of the MR ligand-binding pocket based on its homology with the previously determined crystal structure of the progesterone receptor (PR) LBD [2] (Fig. 1A, B). We found the terminal methyl group of L810 to be in close proximity with the methyl sidechain of A773 on H3, and hypothesized that a novel van der Waals interaction between these residues allowed progesterone-mediated activation of hMR_{S810L} [19].

We tested this model by assaying a series of mutant MRs bearing site specific mutations at these positions. We found that progressive shortening of side-chain length at residue 810 on H5 resulted in a progressive loss of progesterone, but not aldosterone, mediated activation of these mutant receptors. Furthermore, we found evidence for second-site complementation: the loss of activity observed with the shortening of the H5 side chain could be reversed via lengthening of the H3 side chain [19]. These data strongly suggested that progesterone-mediated activation of MR_{S810L} is due at least in part to a novel H3-H5 interaction between leucine 810 and alanine 773 which renders unnecessary the N770-C21-OH interaction otherwise required for MR activity. As A773 lies one turn of the α -helix away from N770 (Fig. 1A), we proposed that the novel H3-H5 interaction might lead to progesterone-mediated receptor activation by bending H3 towards H5.

Confirmation of this proposal required crystallization and analysis of the mutant crystallization. However, crystals of the LBD of MR proved difficult to create due to the insolubility of the native receptor. Progress was ultimately via the introduction of single amino acid substitutions in the LBDs of the receptors to improve their solubility, and the structures of the LBD of MR and MR_{S810L} were recently solved [5,7,8]. Fagart et al. analyzed the structure of MR_{S810L} made with a C910A substitution. They found that L810 lies 4.3Å from A773, a distance too large for vdW interaction, and found instead that the C δ methyl group of L810 lies 3Å from Q776 on H3; they proposed that this contact was crucial to progesterone-mediated activation of MR_{S810L} [5]. In contrast, Bledsoe et al. crystallized MR_{S810L} in the presence of a C808S

substitution. In this model, hydrophobic interaction between L810 and A773 was observed. In addition, while no bend of H3 towards H5 was identified, a kink in H5 in the vicinity of L810 towards H3 was observed, suggesting that the model we proposed may have been partially correct [7]. Whether the differences with respect to L810 interactions noted by these two groups stem from artifacts induced by the differing amino acid substitutions used by these two groups or from other technical differences involved in the formation of the crystal is not clear. Nevertheless, these data all support the general notion that the S810L substitution leads to a novel H3–H5 interaction which is responsible for the increased activity of this receptor.

Conservation of H3–H5 interactions in nuclear receptor

Our studies with the MR_{S810L} receptor suggested that H3–H5 interaction has important effects on the regulation of MR function. This was of interest to us, as we found that the potential for H3–H5 interaction is widely conserved within the SHR family as well as in the NR family. We noted frequent co-conservation of these two residues: SHRs bearing leucine on H5 generally express alanine on H3, while receptors with methionine at the H5 position have glycine on H3 (Fig. 1C). This co-conservation is not limited to humans either, for it is highly conserved in the steroid hormone receptor family in virtually every vertebrate species thus far examined, the lone exception being a glycine to cysteine H3 substitution in chicken and hamster PR that renders the receptor resistant to RU486 antagonism [20].

Additional support for the significance of this interaction comes from data generated from crystal structures of steroid-hormone receptor LBD's in recent years. The corresponding H3 and H5 residues in the human estrogen receptor (A350, L387) and in the progesterone receptor (G722, M759) are in vdW contact [1,2]. Moreover, evidence for this interaction extends beyond the SHR family to the NR family as well; the corresponding residues in receptors as diverse as the retinoid X receptor alpha (A272, L309) and the peroxisome proliferation activation receptor gamma (S317, I354) are in vdW contact as well [21,22]. The wide conservation of this interaction suggested a possible functional role of this interaction as a general determinant of NR activity. To better understand the functional significance of this H3–H5 interaction, we studied these interactions in related steroid receptors.

H5 alteration induces a gain of function in the glucocorticoid receptor

Like hPR, hGR bears methionine (M604) and glycine (G567) at the relevant H5 and H3 positions (Fig. 1C). However, in the recently solved crystal structure of hGR complexed with dexamethasone, M604 is not in vdW contact with G567, and it forms only a weak interaction with the C19 methyl group of dexamethasone (Fig. 2A) [3]. We created a model of hGR based on this structure to analyze H3–H5 interaction. When we substituted leucine for methionine at residue 604 *in silico*, our model predicted that one of the methyl groups of the L604 side chain would be within vdW distance of both the C19 methyl group of dexamethasone (~3.5 Å) and the carbonyl oxygen of the G567 main chain (~4.0 Å, Fig. 2B). Based on our experience with the hMR_{S810L} mutant, we speculated that such a substitution could increase the activity of the receptor. Conversely, when we substituted alanine for glycine on H3 (amino acid 567), our model predicted that the alanine methyl side chain would sterically interfere with the steroid ring and thus decrease receptor activity [23].

We created a series of glucocorticoid receptors bearing mutations at these sites of interest to determine whether alteration of H3–H5 interaction would alter receptor activity as our model predicted. We studied the transcriptional activity of these mutant receptors in Cos7 cells, assessing the ability of the mutant receptors to drive luciferase expression from the mouse mammary tumor virus (MMTV) promoter. Consistent with the predictions of our model, we found that hGR bearing leucine at the H5 position in place of the wild type methionine had increased activity compared to the wild-type receptor. In contrast to what we observed with

hMR_{S810L}, however, the gain-of-function was evidenced by an increase in sensitivity to glucocorticoids without alteration in specificity. We found that hGR_{M604L} is activated by 5–10-fold lower GC concentrations than the wild-type receptor (Fig. 2C, D). Furthermore, the increased activity is not steroid specific, as the receptor showed increased activity with all glucocorticoids tested, indicating that this gain of activity is not dependent on a particular steroid substituent. Interestingly, an hGR_{M604A} mutant also proved to be slightly more active than hGR_{WT}, showing a minor, but statistically significant, increase in transcriptional activity at 0.1 and 1 nM dexamethasone. Again, this increase in transcriptional activity is not dependent on a particular steroid moiety, as it is observed in the presence of all glucocorticoids tested. In contrast, our structural model predicted that alteration of the H3 glycine to alanine would interfere with ligand binding, but not with the H3–H5 interaction observed between L604 and the carbonyl group of residue 567, and our transcriptional activation studies supported this notion. Substitution of alanine for glycine at residue 567 reduces the sensitivity of the receptor by approximately 10-fold, regardless of the H5 residue [23]. Subsequent studies confirmed that the hGR_{M604L} has increased binding affinity for glucocorticoids, an effect which is even more pronounced for corticosteroids which bind GR with lower affinity [23].

Our hGR data are consistent with the notion that a novel vdW interaction between one of the methyl groups of the L604 side chain on H5 and the carbonyl oxygen of G567 on H3 is responsible for the increased activity of hGR_{M604L} (Fig. 2B). As such, the increased activity of this receptor closely correlates with what we previously observed with hMR_{S810L}, that a mutation enabling a new H3–H5 interaction increases receptor activity. While second site substitution of alanine for glycine causes a loss of the observed gain-of-function, the G567A substitution caused a loss of activity in all hGR mutants we tested (Fig. 2C, D), suggesting that the effect of G567A is independent of the H5 residue, and consistent with the model that A567 sterically interferes with steroid binding as opposed to inhibiting a H3–H5 interaction.

H5 alteration induces a gain-of-function in the progesterone receptor

Having identified a critical role of the H3–H5 interaction in regulating receptor activity in hGR, we sought to investigate the role of this interaction in another member of the steroid hormone receptor family, the progesterone receptor (hPR). Like hGR, hPR bears glycine (G722) and methionine (M759), respectively, at the critical H3 and H5 positions; however, these residues are in vdW contact in the crystal structure of the hPR-LBD [2]. To understand the role of this interaction, we tested the activity of progesterone receptors bearing substitutions at the relevant H3 and H5 positions. Interestingly, substitution of leucine for methionine at the H5 position (amino acid 759) conferred a gain-of-function on the receptor. This receptor had substantial constitutive activity, exhibiting 62% of maximal activity in the absence of progesterone ($p < 0.001$ vs. hPR_{WT}, Fig. 3A), and addition of 10 nM progesterone led to a further increase in receptor activity up to 110% of hPR_{WT} activity ($p = \text{NS}$ vs. hPR_{WT}). In addition, we created an A759 mutant to determine the effect of complete disruption of H3–H5 interaction. Interestingly, hPR_{M759A} shares many of the transcriptional properties of hPR_{M759L}. It possesses significant constitutive activity in Cos-7 cells (and HEK293 cells), approximately 40% of hPR_{WT} maximal activity and can be further activated by progesterone to reach maximal activity (Fig. 3B). The constitutive activity of these receptors was inhibited by the progesterone receptor antagonist RU486 [23].

The underlying mechanism for the constitutive activity of hPR_{M759L} and hPR_{M759A} is distinct from that of hGR_{M604L}. Mutant receptors exhibiting constitutive activity have been observed for a number of other members of the NR family [24,25], but the mechanism of this constitutive activity has not always been established. The mutation may allow contacts within the receptor that allow it to fold into an active conformation independent of ligand, or alter receptor interactions with co-activators in a ligand-independent fashion. Alternatively, mutations in the

LBD may allow normally inactive cellular sterols to function as agonists by forming critical contacts with the receptor. For example, cellular fatty acids have been shown to activate the mutant receptor RXR_{F318A}, thus giving this receptor the appearance of constitutive activity [25]. Our finding that RU486 inhibits the constitutive activity of hPR_{M759L} and hPR_{M759A} is consistent with this model, but other models cannot be excluded. Nevertheless, in hPR, as in hGR and hMR, we again show that alteration of the single H5 residue is sufficient to profoundly alter receptor activity and allow creation of a gain-of-function mutant, most likely via alteration of receptor specificity.

The findings described above suggest that interactions between H3 and H5 serve as general regulators of nuclear receptor sensitivity and/or specificity. We have shown here that alterations in H3–H5 residues lead to gain-of-function mutations in hMR, hGR and hPR. Similarly, an ER α receptor bearing a substitution of phenylalanine for alanine at the relevant H3 residue is constitutively active [26]. While loss-of-function mutations in NRs are relatively easy to devise, mutations which increase receptor activity are rare. To our knowledge, the hMR_{S810L} mutation [19] and the hPR_{M759L} (and hPR_{M759A}) mutations are the only known gain-of-function mutations in these receptors. Furthermore, we are aware of only three previously reported gain-of-function mutations in GR [27–29], two of which lie on H3 in close proximity to hGR_{G567}. These findings, coupled with the wide co-conservation of H3–H5 residues in the nuclear receptor family, suggest that the H3–H5 interaction plays a critical role by functioning as a "molecular switch" which regulates the specificity and sensitivity of steroid hormone receptors and perhaps of other NRs as well.

What might be the role of H3–H5 interaction in receptor activity? In the case of hMR_{S810L}, we have noted that the H3–H5 interaction renders the interaction between H3 and the steroid C21 group unnecessary, and we speculated that bending of H3 towards the steroid ligand is a necessary step for MR activation [19]. However, as noted above, Bledsoe et al did not observe a bend in H3, but instead identified a "kink" in H5 in the hMR_{S810L} crystal [7], suggesting that this alteration may be responsible for the changes noted. Given the widespread and almost universal involvement of H3 in the gain-of-function mutations described above, it is tempting to speculate that interactions influencing the bending of H3 or H5 represent a principal mode of steroid hormone receptor activation and that alteration of H3–H5 interaction may represent a general mechanism by which gain-of-function mutations in steroid hormone receptors and perhaps other NRs can be created.

H3–H5 interaction – a common theme in steroid hormone receptor antagonism

As noted above, we identified H3–H5 interaction as a common theme crucial to the sensitivity and specificity of steroid hormone receptors. Of interest, however, these same residues involved play an important role in steroid hormone receptor antagonism as well. In the androgen receptor (AR), alteration of G708 to alanine abolishes the partial agonist activity of steroidal anti-androgens [30]. Similarly, synthetic C-11 substituted spirolactones that normally inhibit MR are potent agonists of an hMR_{A773G} mutant [31]. HPR_{G722A} or hPR_{G722C} mutants have normal receptor activity, but are completely insensitive to the inhibitory action of RU486 [20,32], while the corresponding hGR_{G567A} mutant has been reported to fail to bind RU486 [29]. As hGR, hPR, and AR, which have a glycine at the H3 position, are sensitive to RU486, while hMR possesses an alanine at this position and is insensitive to RU486, it has been proposed that only glycine at this position is permissive for RU486 binding, suggesting that the side chains of other amino acids might sterically interfere with the 11-dimethylaminophenyl group/moiety of RU486 [4,29]. These data imply a major importance of the H3 residue involved in an H3–H5 interaction to steroid hormone receptor antagonist binding. Because of the proximity of H5 to H3 at this site, we proposed that a H5 interaction with either the ligand

(RU486) or the H3 residue might play a role in the activity of receptor antagonists as well. To better understand the amino acid requirements of RU486 mediated inhibition of these receptors, we screened a variety of hPR, hGR, and hMR mutants with mutations at the respective H3 and H5 residues for their response to RU486.

H3–H5 interaction and hPR antagonism

We first confirmed that substitution of alanine for glycine on H3 (residue 722) in hPR renders hPR completely resistant. We further showed, however, that alteration of the H5 position also renders hPR highly resistant to RU486, as 100-fold increased RU486 concentrations are required for receptor inhibition for receptors bearing alanine, glycine, or serine, but not leucine, at the H5 position compared to PR_{WT} (Fig. 4A)[33]. Importantly, RU486 did not stimulate activity in the absence of progesterone in these receptors, indicating that this result is not due to RU486 acting as an agonist. In contrast to the H3 mutations, which inhibit placement of the 11-dimethylaminophenyl moiety of RU486, these data support the proposal that RU486 mediated inhibition of PR is dependent on a constructive interaction between the side chain of residue 759 on H5 and RU486. Moreover, as hPR_{M759S} has no constitutive activity and yet remains resistant to RU486, these data rule out the possibility that competition with an unidentified cellular sterol plays a role in the resistance of H5-altered receptors to RU486.

H3–H5 interaction and hGR antagonism

Our results with hPR suggest that both H3 and H5 residues play an important role in RU486-mediated inhibition. To better understand the effect of H3 and H5 substitutions on antagonist activity, we examined whether mutations at these positions would affect hGR sensitivity to RU486 as well. Although previous reports had suggested hGR containing a substitution of alanine for glycine at the H3 position, hGR_{G567A}, is inactive [29], we found this receptor possessed significant transcriptional activity in the presence of dexamethasone and cortisol, albeit somewhat reduced from that of hGR_{WT} [33], and furthermore, we found that the G567A mutation did not alter RU486 inhibition of hGR; as dexamethasone and cortisol induced activation of hGR_{G567A} were inhibited at similar RU486 concentrations as hGR_{WT} [33]. This stands in sharp contrast to hPR, in which the G722A mutation renders hPR entirely resistant to RU486. This suggests that the underlying stereo-chemistry by which RU486 binds to PR and GR is different, and that the ligand binding pocket of GR is able to adopt a different structure to accommodate the additional moiety on RU486, which is not possible in PR. Taken together, these findings suggest a potential mechanism to alter RU486 in order to create a more specific glucocorticoid antagonist.

It came as a surprise that the hGR_{G567A} mutation is permissive for RU486 binding and activity, because the crystal structure of the hGR-LBD in association with RU486 does not show space for an alanine in this position. However, it must be noted that an F602S mutation was introduced into GR to increase receptor stability in the published crystal structures [3,4]. Given the proximity of the F602S mutation to M604 on H5 (and therefore to G567 on H3), our data suggest that the F602S substitution may have altered the position of H3 with respect to RU486. Consistent with this notion, Kauppi *et al.* noted that the F602S mutation causes a series of side chain movements in the vicinity of residue 602 with respect to the wild-type receptor [4].

Although H3 substitution had no effect on the activity of RU486 on hGR antagonism, we found that receptors with amino acids bearing short, hydrophobic side chains at the H5 position were resistant to RU486, requiring 100-fold increased RU486 concentrations for effective inhibition (Fig. 4B). As with hPR, these data suggest that RU486-mediated inhibition of hGR is dependent on the side chain length of residue 604, and suggests that a van der Waals interaction between RU486 and the side chain of the H5 residue is necessary for efficient RU486 binding. These studies suggest a critical role for H5-ligand interaction in antagonist binding. With

hhPR_{G722A}, it has been suggested that steric hindrance of the alanine side chain with the 11-dimethylaminophenyl group of RU486 is responsible for the lack of efficacy of RU486 [20]. However, steric hindrance cannot explain the insensitivity of hPRs and hGRs containing alanine, serine or glycine to RU486, as the side chains of these residues are substantially smaller than that of the wild-type methionine on H5. We propose that binding of RU486 to steroid hormone receptors is dependent on a constructive interaction between the receptor's H5 residue and RU486, and that this required interaction is lost with substitution of alanine (as well as glycine or serine) for methionine (Fig. 4C).

Conclusion

Altogether, our data suggest that these H3 and H5 residues play a critical role in determining the response of steroid hormone receptors, and perhaps the larger family of nuclear receptors, to agonists and antagonists. By making alterations in these residues gain-of-function mutations have been produced in all steroid hormone receptors thus far tested. With advancements in transgenic and tissue-specific targeting strategies, these may prove to be valuable tools in furthering the study of steroid hormone physiology *in vivo*. Furthermore, our work has demonstrated a critical role of H3–H5 interaction in regulating the sensitivity and/or specificity of all steroid hormone receptors tested to date. Pharmacologic agents which modify or interfere with this interaction, perhaps via the addition of C19 or C11 substitutions on the steroid ring, would be expected to have major effect on the activities of these receptors. Determining the precise structural contacts necessary for steroid hormone receptors ligand-binding will improve our ability to design compounds that regulate steroid hormone receptors and other NRs.

Abbreviations

hPR, human progesterone receptor; hGR, human glucocorticoid receptor; hMR, human mineralocorticoid receptor; LBD, ligand-binding domain; NR, nuclear receptor; H3, helix 3; H5, helix 5; vdW, van der Waals.

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Fig. 1. H3–H5 interaction in progesterone-mediated activation of hMR_{S810L}

(A) Structural model of a portion of the LBD of hMR_{S810L} bound to aldosterone. Based on the crystal structure of the progesterone receptor [2], a model of the hMR LBD was created by substituting hMR-specific residues in the ligand-binding cavity for their corresponding residues in hPR. The side chain of L810 lies in sufficiently close proximity to A773 and the C19 methyl group of the steroid to form van der Waals interactions. (B) Model of hMR_{WT}. The side chain of S810 does not interact with A773. (C) Co-conservation of H3–H5 residues in nuclear receptors. The H3 and H5 amino acid sequences of selected NRs were aligned using Clustal W [34], and the residues corresponding to serine 810 on H5 and alanine 773 on H3 in hMR are highlighted. Receptors bearing leucine at the H5 position frequently have alanine at

the H3 position, while receptors bearing methionine at the H5 position have glycine at the H3 position. MR, mineralocorticoid receptor; ER, Estrogen receptor; ERR2, estrogen-related receptor type 2; RXRG, A, B: Rexinoid receptor gamma, alpha, and beta; AD4BP: adrenal 4 binding protein (also known as SF1, steroidogenic factor 1); PPARG, Peroxisome proliferator-activated receptor gamma; RARA, mouse retinoic acid receptor alpha; GR, glucocorticoid receptor; PR, progesterone receptor; AR, androgen receptor.

Fig. 2. H3–H5 interaction in the GR-LBD

(A) Ribbon drawing of the wild-type hGR-LBD [3]. H3 is colored in purple, H5 in cyan, and dexamethasone in grey. The steroid, the side chain of M604, and the carbonyl oxygen of the G567 main chain are shown as ball-and-stick models and are labeled accordingly. The figure shows that the M604 side chain points away from the ligand, and is too far from G567 to be in vdW contact. (B) Ribbon drawing of the hGR_{M604L} mutant. Unlike M604, the side chain of L604 is in vdW contact with the carbonyl oxygen of the G567 main chain. The structure of the hGR_{M604L} mutant was generated *in silico* by replacing M604 against leucine. (C, D) Increased activity of hGR_{M604L}. The transcriptional activities of the indicated receptors in the presence of cortisol (C) or corticosterone (D) are shown.

Fig. 3. Increased activity of hPR_{M759L}

Transcriptional activity of hPR mutants containing the indicated H3 and H5 substitutions in the presence of progesterone. hPR_{M759L} and hPR_{M759A} each have substantial constitutive activity which is lost in the presence of the second-site G722A substitution on H3.

Fig. 4. A critical role of H5-ligand interaction in RU486 activity

(A) RU486 mediated inhibition of hPR mutants. As previously noted, hPR bearing the G722A H3 substitution is completely resistant to RU486-mediated antagonism. Receptors bearing H5 substitutions with residues containing short hydrophobic sidechains such as alanine (green), glycine, or serine (not shown) are resistant to RU486-mediated antagonism as well. (B) RU486-mediated inhibition of hGR mutants. As with hPR, hGR mutants bearing short hydrophobic sidechains at the H5 position are resistant to RU486-mediated inhibition. (C) A model for the proposed interaction between hPR_{M759} and RU486. Ribbon drawing illustrating the interaction between hPR_{WT} and RU486 based on the crystal structure of the hGR_{F602S}-LBD/RU486 complex [4]. H3 is colored in cyan, H5 in magenta, and RU486 in gray. The steroid, the M759 side-chain, and the carbonyl oxygen of the G722 main-chain are shown as ball-and-stick models and are labeled accordingly. The figure shows that the M759 side-chain is in van der Waals contact with the 11-dimethylaminophenyl moiety of RU486. The figure was prepared with the programs MOLSCRIPT, BOBSCRIPT, and RASTER3D.