3D models of lamprey corticoid receptor complexed with 11-deoxycortisol and deoxycorticosterone

Michael E. Baker[1*], Kayla Y. Uh [2], Paiyuam Asnaashari[2]

[1]Department of Medicine, 0693

[2]Department of Biology

University of California, San Diego

9500 Gilman Drive

La Jolla, CA 92093-0693

Correspondence to:

M. E. Baker

E-mail: mbaker@ucsd.edu

Phone:858-534-8317

Fax: 858-822-0873

Abstract. The serum of Atlantic sea lamprey, a basal vertebrate, contains two corticosteroids, 11-deoxycortisol and deoxycorticosterone. Only 11-deoxycortisol has high affinity [Kd~3 nM] for the corticoid receptor [CR] in lamprey gill cytosol. To investigate the binding of 11-deoxycortisol to the CR, we constructed 3D models of lamprey CR complexed with 11-deoxycortisol and deoxycorticosterone. These 3D models reveal that Leu-220 and Met-299 in lamprey CR have contacts with the 17α-hydroxyl on 11-deoxycortisol. Lamprey CR is the ancestor of the mineralocorticoid receptor [MR] and glucocorticoid receptor [GR]. Unlike human MR and human GR, the 3D model of lamprey CR finds a van der Waals contact between Cys-227 in helix 3 and Met-264 in helix 5. Mutant human MR and GR containing a van der Waals contact between helix 3 and helix 5 display enhanced responses to progesterone and glucocorticoids, respectively. We propose that this interaction was present in the CR and lost during the evolution of the MR and GR, leading to changes in their response to progesterone and corticosteroids, respectively.

1. Introduction

Ancestors of sea lamprey evolved over 500 million years ago, which places lamprey at the base of the vertebrate line. Lamprey's position as a basal vertebrate has motivated studies of lamprey to understand the evolution of vertebrates [1-3]. Sea lamprey (*Petromyzon marinus*) contains orthologs of the corticoid receptor [CR], progesterone receptor [PR] and estrogen

receptor [ER] [4], which makes lamprey an important animal for understanding the evolution of vertebrate adrenal and sex steroid receptors [5-14].

Previously, we constructed 3D models of lamprey ER with various estrogens [15] and lamprey PR with various progestins [16]. These 3D models revealed that many structural interactions were conserved between estrogens in lamprey ER and human ER, and between progestins in lamprey PR and human PR. However, both lamprey ER and PR also contained some unique features that appeared to be important in the binding of 15α-hydroxy-estradiol to lamprey ER and 15α-hydroxy-progesterone to lamprey PR. The success of these previous studies motivated us to examine the binding of steroids to lamprey CR, which is the ancestor of the mineralocorticoid receptor [MR] and glucocorticoid receptor [GR] [4, 7, 17].

In addition to our interest in understanding how steroids bind to lamprey CR, we also were motivated by intriguing data from two reports on steroid binding to lamprey CR. In the first report, 100 nM of cortisol, corticosterone, 11-deoxycortisol, 11-deoxycorticosterone (DOC) or aldosterone activated transcription in mammalian cells of the ligand-binding domain of lamprey CR fused to a GAL4-DNA-binding domain [7]. At concentrations below 10 nM, only aldosterone, corticosterone and DOC were transcriptionally active.

In the second report, Close et al. [18] found that 11-deoxycortisol and DOC were the two endogenous corticosteroids in lamprey serum. Neither cortisol nor corticosterone were present in lamprey serum. Further *in vitro* binding studies of native CR in lamprey gill cytosol revealed that only 11-deoxycortisol had high affinity [Kd~3 nM] for lamprey CR. DOC, a mineralocorticoid, bound to CR with a substantially lower affinity than 11-deoxycortisol [18]. Moreover, neither cortisol, corticosterone nor progesterone bound to lamprey CR. These data and the evidence that the synthesis of 11-deoxycortisol in lamprey was stimulated by stress and that 11-deoxycortisol down-regulated synthesis of sex steroids and up-regulated gill Na^+K^+-ATPase prompted Close et al. [18] to propose that 11-deoxycortisol is the endogenous corticosteroid in sea lamprey.

With these reports [7, 18] in mind, we constructed 3D models of lamprey CR complexed with 11-deoxycortisol and with DOC, the other corticosteroid in lamprey serum. We also constructed a 3D model of human MR with 11-deoxycortisol , which along with the crystal structure of the human MR with DOC [19], were compared with the 3D models of lamprey CR with 11-deoxycortisol and DOC. Analysis of our 3D models of lamprey CR and human MR

with 11-deoxycortisol indicates that its 17α-hydroxyl has van der Waals contacts with Leu-220 and Met-299 on lamprey CR and with the corresponding Leu-766 and Met-845 in human MR. In contrast, DOC does not contact either Leu-220 in lamprey CR or Leu-766 in human MR.

During our analysis of the 3D model of lamprey CR, we uncovered an unexpected van der Waals contact between Cys-227 in helix 3 and Met-264 in helix 5 in lamprey CR. This van der Waals contact is absent between the corresponding residues in helix 3 and helix 5 in human MR [Ala-773, Ser-810] [20] and human GR [Gly-567, Met-604] [21]. The pathophysiological importance of this van der Waals contact was demonstrated by Geller et al.[20], who found that a Ser-810 to Leu mutation in human MR results in an interaction between Leu-810 and Ala-773, which changes progesterone from a mineralocorticoid antagonist to an agonist. Subsequently, Zhang et al. [21] showed that for the GR, mutation of Met-604 to leucine in helix 5 leads to a van der Waals contact with Gly-567 and an increased response to glucocorticoids. The presence of this helix 3-helix 5 interaction in the 3D model of lamprey CR and its absence in human MR and human GR leads us to propose that this interaction was present early in vertebrate evolution and lost later in the evolution of the MR and GR with important consequences for their response to 3keto-steroids.

2. Experimental

2.1. Construction of 3D Models

The 3D structure of human MR co-crystalized with corticosterone [PDB:**2A3I**] [22] was used as a template for constructing the 3D model of lamprey CR [Genbank:**AAK20930**]. The sequences of the steroid-binding domain of lamprey CR and human MR are 67% identical, and after including conservative replacements [e.g. arginine/lysine, aspartic acid/glutamic acid], there are 76% positives with no gaps [Figure 1]. This strong similarity between lamprey CR and its template gives us confidence in the accuracy of our lamprey 3D model [23]. We used the Homology option in Insight II to construct a 3D model of lamprey CR in the PDB format.

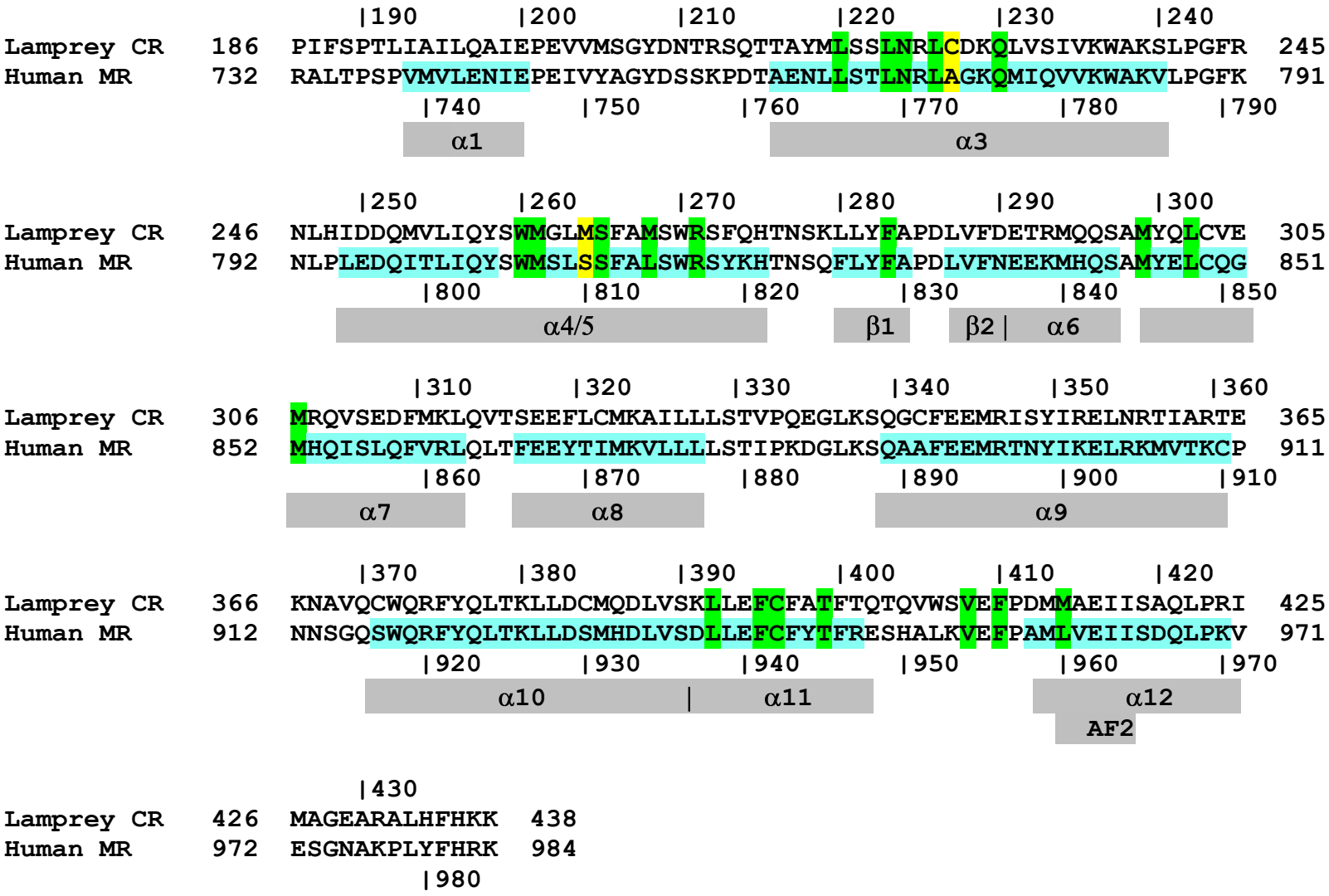

Figure 1. Alignment of lamprey CR with human MR. α-helices and β-strands from the crystal structure of human MR [PDB: **2A3I**] are shown in blue and notated below the alignment. Residues in human MR that contact corticosterone are shown in green[22]. The residues involved in the interaction between helix 3 and helix 5 in human MR in the 3dmodel of lamprey CR are shown in yellow.

After we obtained the apo-3D model of lamprey CR, we inserted corticosterone into lamprey CR from human MR, by overlapping lamprey CR with human MR and extracting corticosterone from human MR for insertion into lamprey CR using the Biopolymer option in Insight II. Corticosterone in lamprey CR and human MR was converted to 11-deoxycortisol by adding the 17α-hydroxyl and removing the 11β-hydroxyl. DOC was constructed from corticosterone by removal of the 11β-hydroxyl. We used the crystal structure of human MR with DOC [PDB:**2AA7**] [19] to analyze the binding of DOC to the MR.

We refined the structure of lamprey CR with 11-deoxycortisol and DOC and human MR with 11-deoxycortisol using Discover 3 in Insight II. For this energy minimization step, Discover 3 was run for 10,000 iterations, using the CVFF force field and a distant dependent dielectric constant of 2 to approximate for water in the protein.

3. Results

In Figure 2, we show the overlap of the Cα-backbones of our 3D model of lamprey CR and the crystal structure of human MR. The root mean square deviation [RMSD] of their Cα chains is 1.2 Å, which indicates good overall similarity between our 3D model of lamprey CR and human MR.

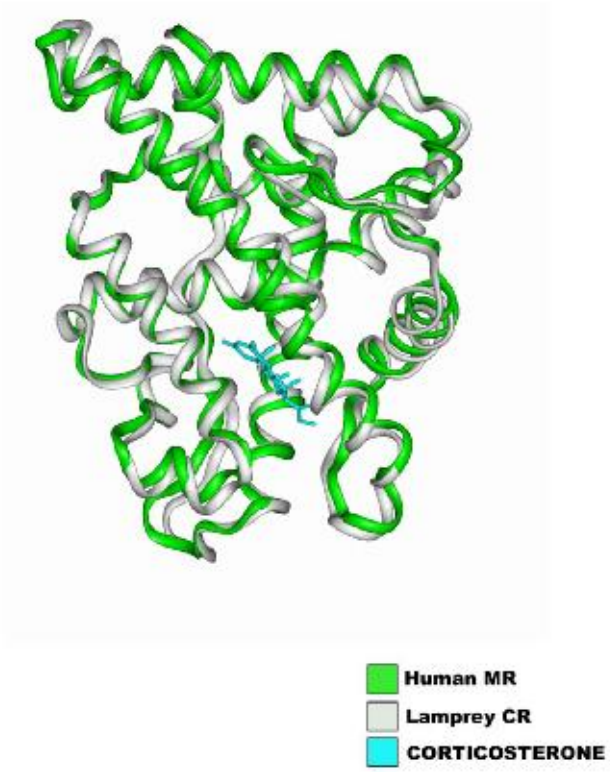

Figure 2. Overlap of 3D models of lamprey CR with human MR. The 3D model of lamprey CR with 11-deoxycortisol was superimposed on human MR complexed with corticosterone [PDB:**2A3I]**. There is excellent overlap. The root mean square deviation between the Cα backbone of human MR and lamprey CR is 1.2Å.

3.1. Analysis of 11-Deoxycortisol binding to the 3D model of lamprey CR

The interactions of key residues in our 3D model of lamprey CR with 11-deoxycortisol are shown in Figure 3A. Gln-230, Arg-271, Phe-283, Cys-227 and Met-264 stabilize the A ring on 11-deoxycortisol. The C3-ketone on the A ring is 3.2Å from Nε2 on Gln-230 and 3.1Å from Nη2 on Arg-271. Nε on Arg-271 is 3.0Å from the backbone oxygen on Phe-283. Cδ2 on Phe-283 is 4.2Å from the C3 ketone. Sγ on Cys-227 and Sδ2 on Met-264 are 4Å and 3.6Å, respectively from C19. Asn-224, Cys-396, Phe-395 and Thr-399 stabilize the D ring on 11-deoxycortisol. Nδ2 on Asn-224 is 3.1Å from the C21 hydroxyl; Cδ1 on Phe-395 is 3.2Å from the C20 ketone; Sγ on Cys-396 is 3.7Å from C18 and Oγ on Thr-399 is 2.9Å from the C20 ketone and 2.8Å from the C21 hydroxyl on 11-deoxycortisol.

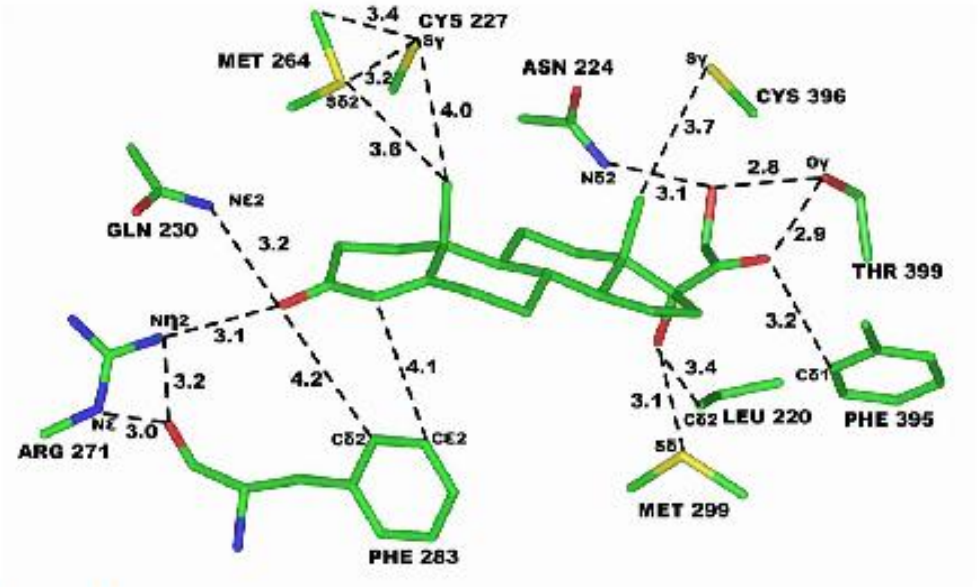

Figure 3A

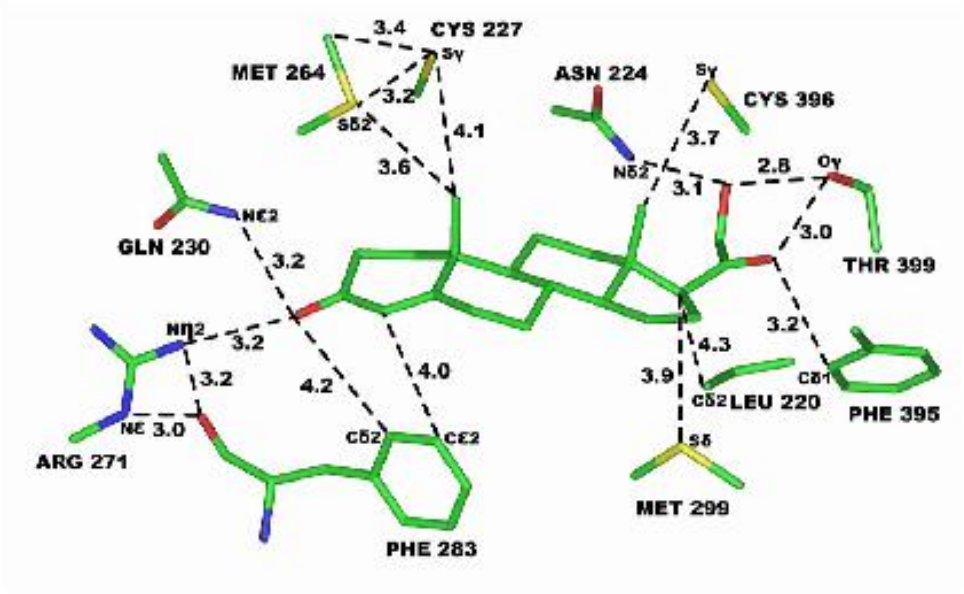

Figure 3B

Figure 3. Interaction of 11-deoxycortisol and DOC with the 3D model lamprey CR. With the exception of the 17α-hydroxyl on 11-deoxycortisol, both 11-deoxycortisol and DOC have similar stabilizing contacts with lamprey CR.

A. Interaction of 11-deoxycortisol with lamprey CR. Gln-230, Arg-271, Phe283, Cys-227 and Met-264 in lamprey CR have stabilizing contacts with the A ring of 11-deoxycortisol. Asn-224, Cys-396, Thr-399 and Phe-395 stabilize the D-ring. Leu-220 and Met-299 and have

van der Waals contacts with the 17α-hydroxyl on 11-deoxycortisol. Cys-227 and Met-264 have a van der Waals contact.

B. Interaction of DOC with lamprey CR. The stabilizing contacts between DOC and lamprey CR are similar to that for binding of 11-deoxycortisol to lamprey CR. Met-299 has a van der Waals contact with C16 on DOC. Leu-220 is distant from DOC. Cys-227 and Met-264 have a van der Waals contact.

The 17α-hydroxyl on 11-deoxycortisol has van der Waals contacts with Leu-220 and Met-299. In addition, there is a van der Waals contact between Sγ on Cys-227 in helix 3 and Sδ on Met-264 on helix 5. As will be discussed later, this helix 3-helix 5 interaction is important in the response to steroids by the MR, GR and PR [24].

3.2. Analysis of DOC binding to the 3D model of lamprey CR

The interactions of key residues in our 3D model of lamprey CR with DOC are shown in Figure 3B. The distances between these residues and the A and D rings on DOC are similar to those found for 11-deoxycortisol and lamprey CR. An important exception is that Cδ2 on Leu-220 is 4.3Å from DOC.

3.3. Analysis of 11-Deoxycortisol and DOC binding to human MR

In Figure 4, we present an analysis of the binding of 11-deoxycortisol and DOC to human MR for comparison with 3D models of lamprey CR [Figure 3]. Our 3D model of human MR with 11-deoxycortisol [Figure 4A] shows that human MR has similar contacts with 11-deoxycortisol as found our 3D model of lamprey CR [Figure 3A]. For example, Gln-776, Arg-817 are 3.5Å and 2.7Å, respectively, from the C3-ketone on 11-deoxycortisol, and Thr-945 is 3Å from C20 ketone and C21 hydroxyl on 11-deoxycortisol. Of importance is that Sδ2 on Met-845 is 2.6Å and Cδ2 on Leu-766 is 3.3Å from the 17α-hydroxyl on 11-deoxycortisol.

Figure 4B is based on the solved crystal structure of human MR with DOC [PDB:**2AA7**] [19]. The distances between Arg-817, Gln-776 and Phe-829 in the human MR and the C3 ketone in DOC are similar to that for DOC in the 3D model of lamprey CR [Figure 3B]. The D ring on DOC is stabilized by Asn-773, Cys-942, Thr-9454 and Phe-941 in human MR.

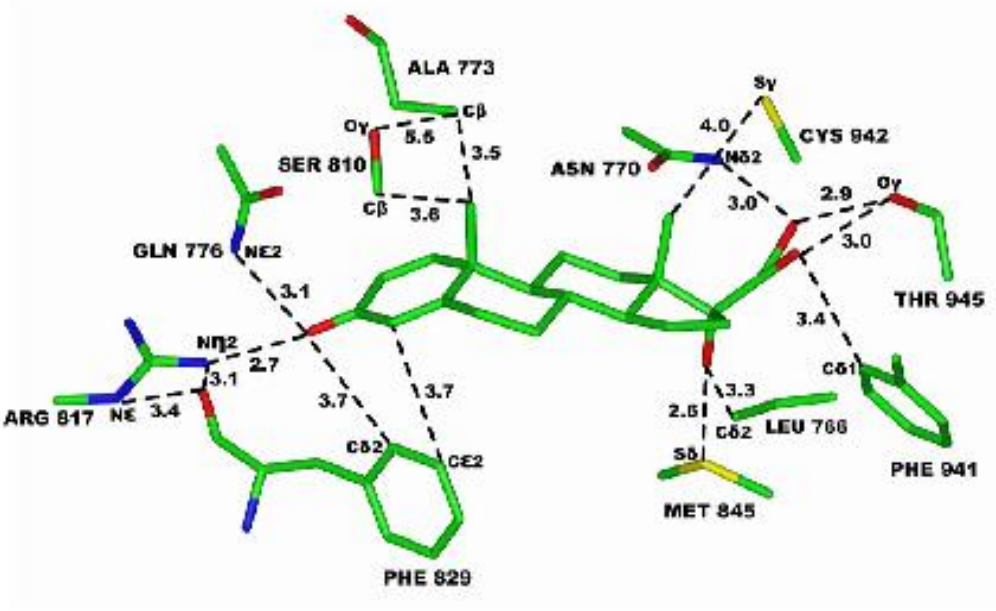

Figure 4A

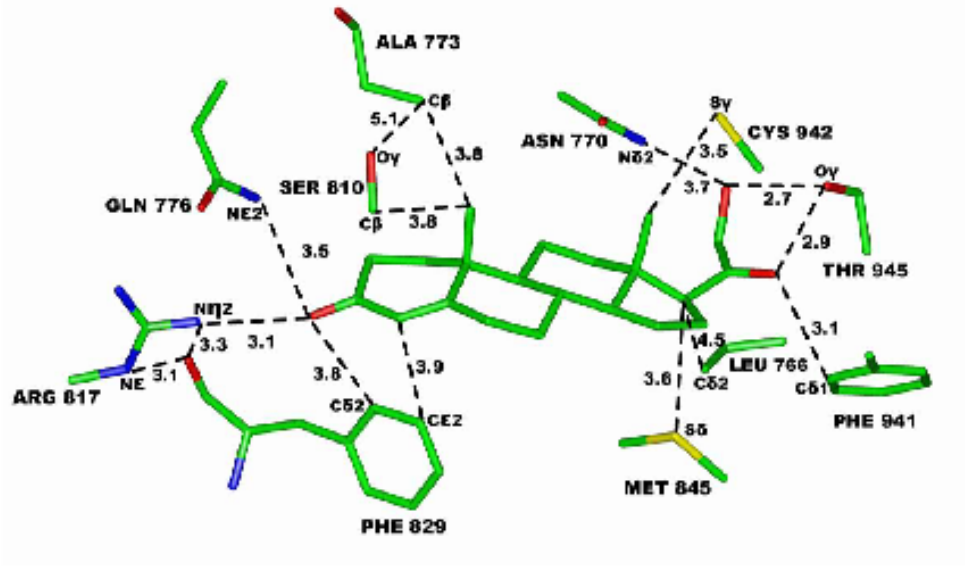

Figure 4B

Figure 4. Interaction of DOC and 11-deoxycortisol with human MR.
With the exception of the 17α-hydroxyl on 11-deoxycortisol, both 11-deoxycortisol and DOC have similar stabilizing contacts with human MR.

A. Interaction of 11-deoxycortisol with human MR. Gln-776, Arg-817, Phe-829, Ala-773, Ser-810 in human MR have stabilizing interactions with the A ring of 11-deoxycortisol. Asn-773, Cys-942, Thr-945 and Phe-941 stabilize the D-ring. Leu-220 and Met-845 and have

van der Waals contacts with the 17α-hydroxyl on 11-deoxycortisol. The distance between Cβ on Ala-773 and Oγ on Ser-810 is 5.5Å.

B. Interaction of DOC with human MR [19]. Gln-776, Arg-817, Phe-829, Ala-773, Ser-810 in human MR have stabilizing interactions with the A ring of 11-deoxycortisol. Asn-773, Cys-942, Thr-945, Phe-941 and Leu-x stabilize the D-ring. Leu-220 and Met-845 and have van der Waals contacts with the 17α-hydroxyl on 11-deoxycortisol. The distance between Cβ on Ala-773 and Oγ on Ser-810 is 5.1Å.

The distances between steroids and human MR, shown in Figure 4, are similar to that in the crystal structures of human MR complexed with corticosterone [PDB:**2A3I**][22] [Figure 5A] and aldosterone [PDB: **2AA2**] [19] [Figure 5B].

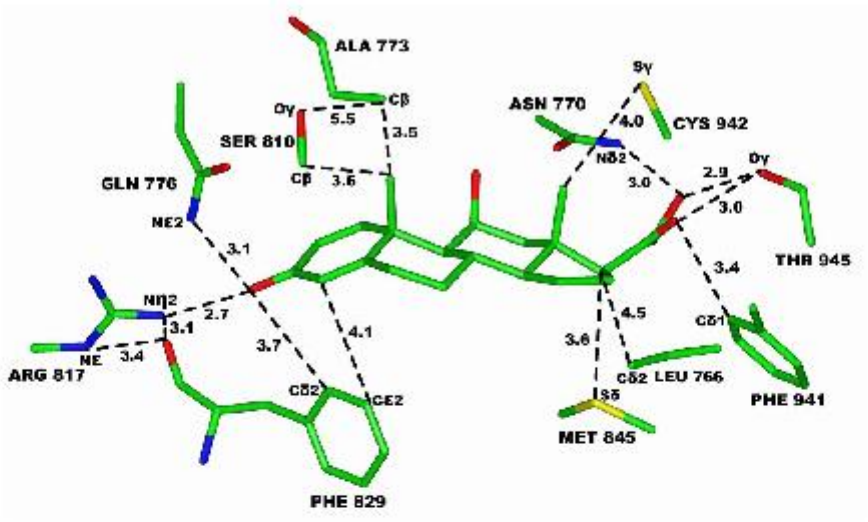

Figure 5A

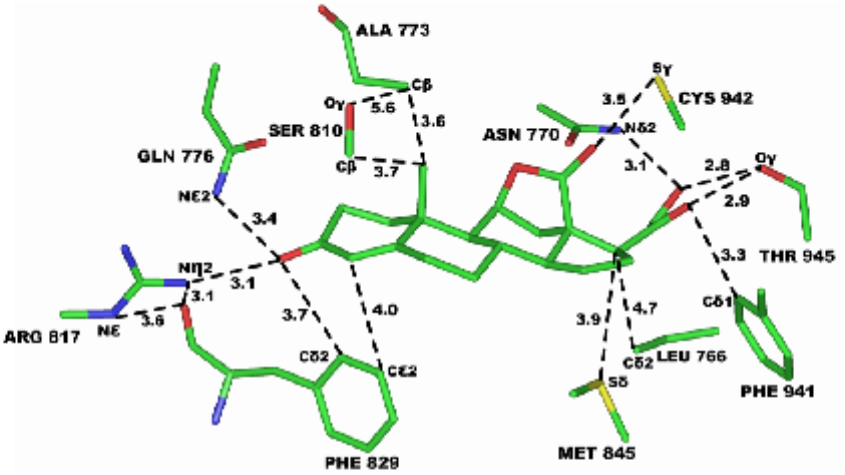

Figure 5B

Figure 5. Interaction of corticosterone and aldosterone with human MR.
A. Key amino acids in the steroid binding site of human MR with corticosterone [24]. Stabilizing contacts found between corticosterone and human MR are similar to that for binding of 11-deoxycortisol and DOC to human MR.
B. Key amino acids in the steroid binding site of human MR with aldosterone [19]. The stabilizing contacts between aldosterone and human MR are similar to that for binding of 11-deoxycortisol and DOC to human MR.

3.4. Lamprey CR contains a stabilizing interaction between helix 3 and helix 5

During our analysis of the 3D model of lamprey CR with 11-deoxycortisol and DOC, we discovered that Sγ on Cys-227 in helix 3 has a contact with Sδ on Met-264 in helix 5 [Figures 3 and 6A]. The corresponding residues in human MR are Ala-773 and Ser-810. A contact between Ala-773 and Ser-810 is absent in the crystal structure of human MR complexed with DOC [19] [Figure 4B], corticosterone [22] [5A], aldosterone [19] [Figure 5B] and in our 3D model of human MR with 11-deoxycortisol [Figures 4A and 6B]. The absence of this contact in the MR is important as Geller et al. [20] found that mutation of Ser-810 to leucine yielded a mutant human MR that was activated by progesterone, which otherwise is an antagonist of wild-type MR. In the Leu-810 mutant MR, Leu-810 interacts with Ala-773.

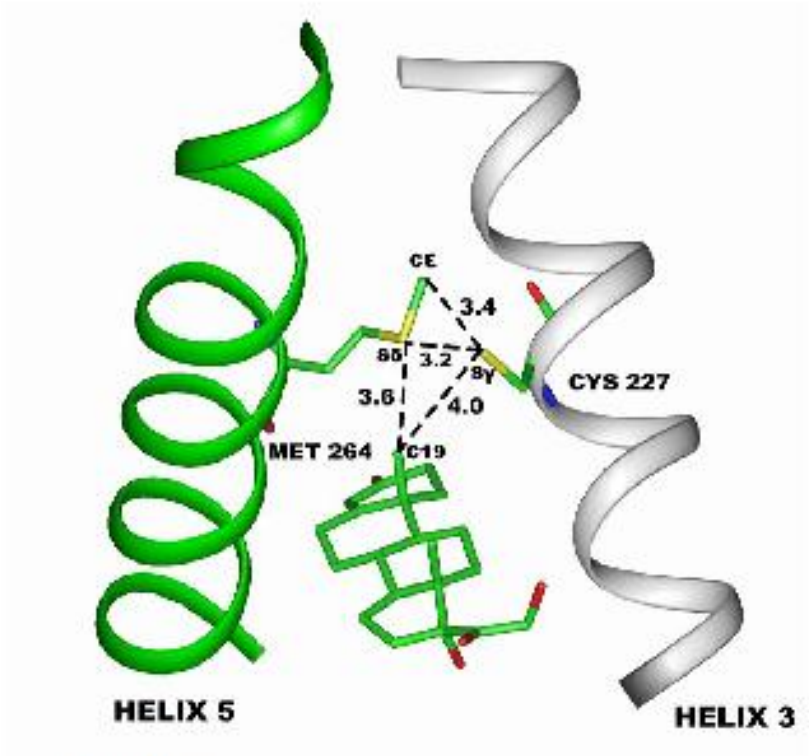

Figure 6A

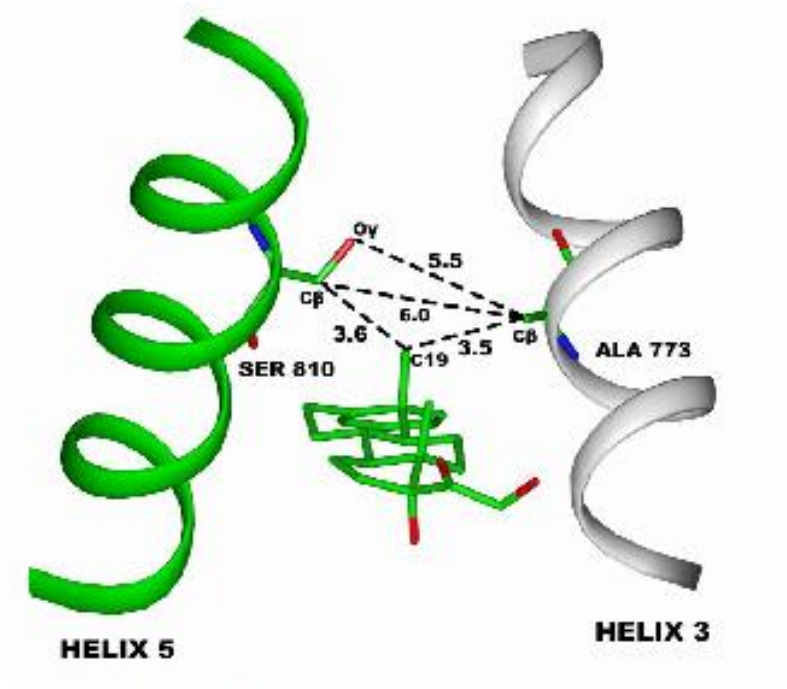

Figure 6B

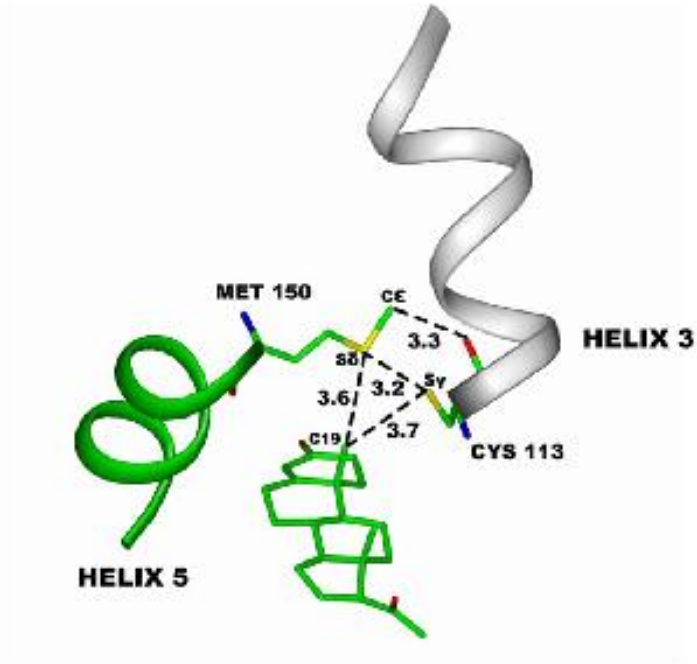

Figure 6C

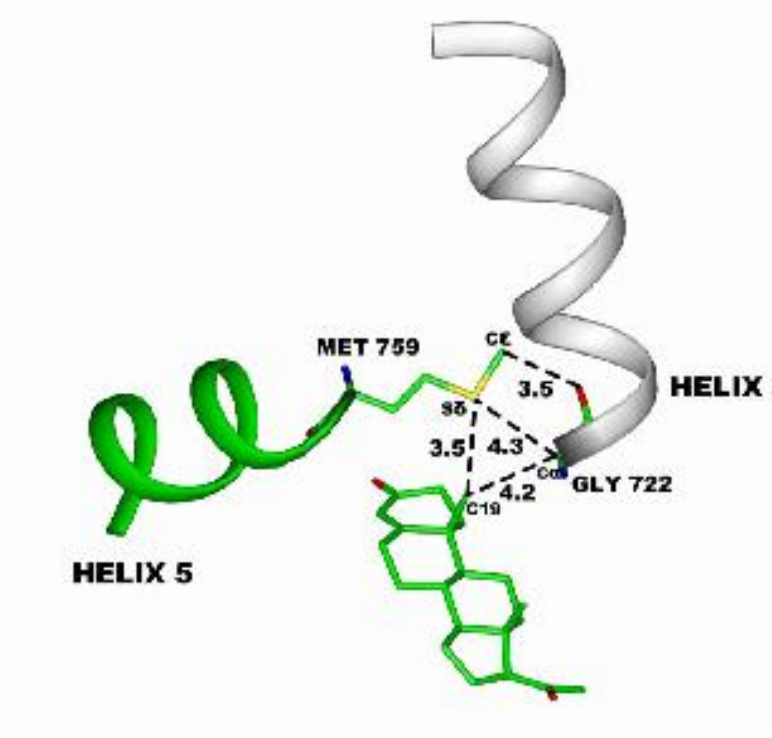

Figure 6D

Figure 6. Analysis of interactions between helix 3 and helix 5 in lamprey CR, lamprey PR, human MR and human PR.

3D models of lamprey CR and lamprey PR identify a contact between helix 3 and helix 5 that is absent in human MR and GR and present in human PR.

A. In the 3D model of lamprey CR with 11-deoxycortisol, Cys-227 on helix 3 and Met-264 on helix 5 are 3.2Å distant.

B. In the 3D model of human MR with 11-deoxycortisol, Ala-773 on helix 3 and Ser-810 on helix 5 are 5.5Å distant.

C. In the 3D model of lamprey PR with progesterone, Cys-113 on helix 3 and Met-150 on helix 5 are 3.3Å distant.

D. In human PR with progesterone, Gly722 on helix 3 and Met 756 on helix 5 are 3.5Å distant.

4. Discussion

The evidence from an analysis of the amino acid sequence of lamprey CR that it is closest to the MR [7] motivated the construction of a 3D model of lamprey CR for comparison with the crystal structure of human MR to elucidate the evolution of the structure of the steroid binding domain in the MR. The intriguing findings of Close et al. [18] that 11-deoxycortisol and DOC are the only corticosteroids in lamprey serum; that only 11-deoxycortisol has high affinity

binding to lamprey gill cytosol, and that 11-deoxycortisol up-regulated gill Na^+K^+-ATPase also motivated the construction of 3D models of lamprey CR with 11-deoxycortisol and DOC.

4.1. Comparison corticosteroid binding in the 3D model of lamprey CR and human MR

Overall, an analysis of the binding of 11-deoxycortisol and DOC to lamprey CR [Figure 3] and human MR [Figure 4] reveals that key interactions of the A ring of these two steroids with lamprey CR and human MR are conserved, as are many interactions with the D ring.

Of relevance for binding of 11-deoxycortisol to lamprey CR are the contacts of 17α-hydroxyl on 11-deoxycortisol with Sδ on Met-299 and Cδ2 on Leu-220 in lamprey CR [Figure 3A]. The corresponding amino acids in the 3D model of human MR with 11-deoxycortisol are Met-845 and Leu-766, which also have van der Waals contacts with the 17α-hydroxyl group [Figure 4A]. These stabilizing contacts in human MR are consistent with transcriptional activation of human MR by1 nM 11-deoxycortisol [20].

Regarding binding of DOC to lamprey CR, Leu-220 is too distant for a van der Waals contact with DOC [Figure 3B]. In the human MR, Leu-766 also is distant from DOC [Figure 4B] [19]. Nevertheless, DOC has a nM affinity for human MR [25-26]. Thus, it is unlikely that the absence of a stabilizing contact between Leu-220 and DOC explains its low affinity for the CR in lamprey gill cytosol [18]. The lower affinity of DOC for lamprey CR may be due to post-transcriptional modifications of the CR that are specific to lamprey, the absence of inter-domain interactions that are present in human MR [27] or to apparently trivial sequence differences outside of the steroid-binding domain in the CR, as has been found for ligand binding to the estrogen receptor [28].

Interestingly, transcriptional studies in mammalian cells of the ligand-binding domain of lamprey CR fused to a GAL4-DNA-binding domain indicate that DOC has a Kd of about 4 nM for lamprey CR and 11-deoxycortisol has a Kd of about 80 nM [7]. As noted previously [18], these data showing high affinity of DOC and lower affinity of 11-deoxycortisol for lamprey CR may be due to differences between transcriptional assays in mammalian cells and hormone-binding assays in lamprey gill cytosol or to differences in inter-domain interactions in the lamprey CR and human MR [27] or to post-translational modifications of the receptors or to specific co-regulators in lamprey and human cells [29].

4.2. Evolutionary Implications

Various hypotheses have been advanced to explain the origins of the MR and its relationship to the GR and other steroid receptors [7, 11, 16-17, 30-31]. Lamprey and hagfish, two basal vertebrates, contain a CR that is closer to the MR than to the GR [7], which supports the hypothesis that the MR is ancestral to the GR [7, 11, 17, 30, 32]. Lamprey also contains a progesterone receptor [PR], leaving unanswered the identity of the common ancestor of the CR and PR and the steroid[s] that regulated the ancestral 3keto-steroid receptor [16, 33-34].

Our unexpected finding that the 3D model of lamprey CR predicts the presence of a van der Waals interaction between Cys-227 in helix 3 and Met-264 in helix 5 in lamprey CR provides an important insight into the evolution of steroid specificity for the MR and GR. In human MR, the corresponding Ala-773 and Ser-810 lack a van der Waals contact between helix 3 and helix 5 [19, 22] [Figures 5-7]. Geller et al. [20] found that replacing Ser-810 with leucine yielded a mutant MR in which there was an interaction between helix 3 and helix 5, and this interaction changed progesterone from an MR antagonist to an agonist. Moreover, 1 nM 19-norprogesterone, pregnenolone and 17α-OH-progesterone became activators of gene transcription of the Leu-810 mutant MR in Cos-7 cells. Interestingly, cortisone, which does not bind to human MR, is a transcriptional activator of the Leu-810 mutant MR [35]. Further studies with the human GR [21] showed that mutation of Met-604 in helix 5 to leucine provided an interaction with Gly-567 on helix 3, which increased the response to glucocorticoids. Together, these studies established the importance of the helix 3-helix 5 interaction in regulating the specificity and responses to steroids in the human MR and GR.

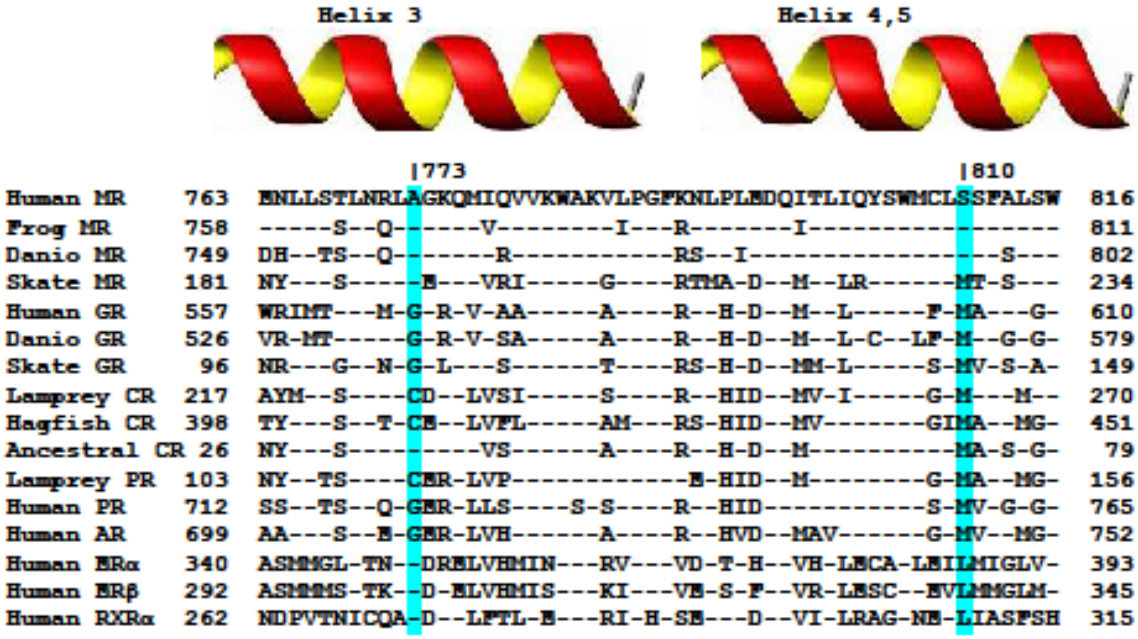

Figure 7. Sequences in nuclear receptors that correspond to helix 3 and helix 5.
Sequences from various steroid receptors that align with helix 3 and helix 5 in the human MR are shown [also see [11, 21]]. Residues involved in the interaction between helix 3 and helix 5 are shown in blue. Residues in other species that are identical to those in human MR are denoted with a -.
Residues corresponding to Ala-773 on human MR are cysteine in lamprey CR, hagfish CR and lamprey PR, an alanine in skate MR, frog MR and Danio MR. At this position, skate GR contains a glycine, as do Frog GR, Danio GR, human PR and AR. An alanine is in this position in human ERα, ERβ and RXRα, [11, 21].
Residues corresponding to human Ser-810 on human MR are methionine in lamprey CR, lamprey PR, hagfish CR, skate MR, skate GR, frog MR, frog GR, human PR and AR. A leucine is in this position in human ERα, ERβ and RXRα, [11, 21].

Re-examination of our 3D model of lamprey PR with progesterone indicates that it predicts the presence of a van der Waals contact between Cys-113 in helix 3 and Met-150 in helix 5 in lamprey PR [16] [Figure 6C]. In human PR, Gly-722 in helix 3 and Met-759 in helix 5 have a van der Waals contact. Interestingly, Zhang et al. [21] found that replacing Gly-722 in helix 3 with alanine yielded a mutant human PR with constitutive activity. This provided

additional evidence that the interaction between helix 3 and helix 5 is important in transcriptional activation of steroid receptors.

The evidence from our 3D models of lamprey CR and PR showing a key helix 3-helix 5 contact, and the conservation of the corresponding cysteine and methionine in hagfish CR [Figure 7] suggests that the helix 3-helix 5 interaction was conserved in the common ancestor of lamprey and hagfish. Moreover, other analyses suggest that this contact is ancient, as it is found in the ER, RXR and other nuclear receptors [Figure 7] [11, 21, 24]. However, unequivocal proof of the primordial origin of the helix 3-helix 5 interaction will require crystal structures of lamprey CR with various corticosteroids.

Interestingly, skate MR appears to be an evolutionary intermediate in the loss of the helix 3-helix 5 interaction in vertebrate MRs. Skate MR has an alanine at the position corresponding to lamprey Cys-227, while skate MR contains a methionine corresponding to lamprey Met-264 in helix 5. Based on studies showing that the human Ser810Met mutant MR responds to 19-nor-progesterone [20], skate MR would be expected to retain an interaction between helix 3 and helix 5. It appears that this interaction was lost later, when methionine was replaced by serine in helix 5 in teleosts and land vertebrates [Figure 7] [20, 30].

Based on the changes in the response of human MR and human GR to steroids due to mutations in helix 3 and helix 5 [20-21], it appears that the loss of this interaction was important in the evolution of corticosteroid specificity in vertebrate MR and GR, although mutations at other positions in the MR and GR also appear to be important [7, 17, 36].

References

[1] Doolittle RF, Jiang Y, Nand J. Genomic evidence for a simpler clotting scheme in jawless vertebrates. J Mol Evol. 2008;66:185-96.
[2] Osorio J, Retaux S. The lamprey in evolutionary studies. Dev Genes Evol. 2008;218:221-35.
[3] Sauka-Spengler T, Bronner-Fraser M. Insights from a sea lamprey into the evolution of neural crest gene regulatory network. Biol Bull. 2008;214:303-14.
[4] Thornton JW. Evolution of vertebrate steroid receptors from an ancestral estrogen receptor by ligand exploitation and serial genome expansions. Proc Natl Acad Sci U S A. 2001;98:5671-6.
[5] Escriva H, Delaunay F, Laudet V. Ligand binding and nuclear receptor evolution. Bioessays. 2000;22:717-27.
[6] Baker ME. Evolution of adrenal and sex steroid action in vertebrates: a ligand-based mechanism for complexity. Bioessays. 2003;25:396-400.

[7] Bridgham JT, Carroll SM, Thornton JW. Evolution of hormone-receptor complexity by molecular exploitation. Science. 2006;312:97-101.
[8] Baker ME. Evolution of metamorphosis: role of environment on expression of mutant nuclear receptors and other signal-transduciton proteins. Integrative and Comparative Biology. 2006;46:808-14.
[9] Paris M, Pettersson K, Schubert M, Bertrand S, Pongratz I, Escriva H, et al. An amphioxus orthologue of the estrogen receptor that does not bind estradiol: insights into estrogen receptor evolution. BMC Evol Biol. 2008;8:219.
[10] Markov GV, Tavares R, Dauphin-Villemant C, Demeneix BA, Baker ME, Laudet V. Independent elaboration of steroid hormone signaling pathways in metazoans. Proc Natl Acad Sci U S A. 2009;106:11913-8.
[11] Hu X, Funder JW. The evolution of mineralocorticoid receptors. Mol Endocrinol. 2006;20:1471-8.
[12] Eick GN, Thornton JW. Evolution of steroid receptors from an estrogen-sensitive ancestral receptor. Mol Cell Endocrinol. 2011;334:31-8.
[13] Baker ME. Origin and diversification of steroids: co-evolution of enzymes and nuclear receptors. Mol Cell Endocrinol. 2011;334:14-20.
[14] Markov GV, Laudet V. Origin and evolution of the ligand-binding ability of nuclear receptors. Mol Cell Endocrinol. 2011;334:21-30.
[15] Baker ME, Chang DJ, Chandsawangbhuwana C. 3D model of lamprey estrogen receptor with estradiol and 15alpha-hydroxy-estradiol. PLoS One. 2009;4:e6038.
[16] Baker ME, Asnaashari P, Chang DJ, McDonnell S. 3D models of lamprey progesterone receptor complexed with progesterone, 7alpha-hydroxy-progesterone and 15alpha-hydroxy-progesterone. Steroids. 2011;76:169-76.
[17] Baker ME, Chandsawangbhuwana C, Ollikainen N. Structural analysis of the evolution of steroid specificity in the mineralocorticoid and glucocorticoid receptors. BMC Evol Biol. 2007;7:24.
[18] Close DA, Yun SS, McCormick SD, Wildbill AJ, Li W. 11-deoxycortisol is a corticosteroid hormone in the lamprey. Proc Natl Acad Sci U S A. 2010;107:13942-7.
[19] Bledsoe RK, Madauss KP, Holt JA, Apolito CJ, Lambert MH, Pearce KH, et al. A ligand-mediated hydrogen bond network required for the activation of the mineralocorticoid receptor. J Biol Chem. 2005;280:31283-93.
[20] Geller DS, Farhi A, Pinkerton N, Fradley M, Moritz M, Spitzer A, et al. Activating mineralocorticoid receptor mutation in hypertension exacerbated by pregnancy. Science. 2000;289:119-23.
[21] Zhang J, Simisky J, Tsai FT, Geller DS. A critical role of helix 3-helix 5 interaction in steroid hormone receptor function. Proc Natl Acad Sci U S A. 2005;102:2707-12.
[22] Li Y, Suino K, Daugherty J, Xu HE. Structural and biochemical mechanisms for the specificity of hormone binding and coactivator assembly by mineralocorticoid receptor. Mol Cell. 2005;19:367-80.

[23] Sali A, Blundell TL. Comparative protein modelling by satisfaction of spatial restraints. J Mol Biol. 1993;234:779-815.
[24] Zhang J, Geller DS. Helix 3-helix 5 interactions in steroid hormone receptor function. J Steroid Biochem Mol Biol. 2008;109:279-85.
[25] Arriza JL, Weinberger C, Cerelli G, Glaser TM, Handelin BL, Housman DE, et al. Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. Science. 1987;237:268-75.
[26] Hellal-Levy C, Couette B, Fagart J, Souque A, Gomez-Sanchez C, Rafestin-Oblin M. Specific hydroxylations determine selective corticosteroid recognition by human glucocorticoid and mineralocorticoid receptors. FEBS Lett. 1999;464:9-13.
[27] Pippal JB, Yao Y, Rogerson FM, Fuller PJ. Structural and functional characterization of the interdomain interaction in the mineralocorticoid receptor. Mol Endocrinol. 2009;23:1360-70.
[28] Chen Z, Zhao H. Rapid creation of a novel protein function by in vitro coevolution. J Mol Biol. 2005;348:1273-82.
[29] Viengchareun S, Le Menuet D, Martinerie L, Munier M, Pascual-Le Tallec L, Lombes M. The mineralocorticoid receptor: insights into its molecular and (patho)physiological biology. Nucl Recept Signal. 2007;5:e012.
[30] Kassahn KS, Ragan MA, Funder JW. Mineralocorticoid receptors: evolutionary and pathophysiological considerations. Endocrinology. 2011;152:1883-90.
[31] Carroll SM, Bridgham JT, Thornton JW. Evolution of hormone signaling in elasmobranchs by exploitation of promiscuous receptors. Mol Biol Evol. 2008;25:2643-52.
[32] Ortlund EA, Bridgham JT, Redinbo MR, Thornton JW. Crystal structure of an ancient protein: evolution by conformational epistasis. Science. 2007;317:1544-8.
[33] Lowartz SM, Renaud RL, Beamish FW, Leatherland JF. Evidence for 15alpha- and 7alpha-hydroxylase activity in gonadal tissue of the early-life stages of sea lampreys, Petromyzon marinus. Comp Biochem Physiol B Biochem Mol Biol. 2004;138:119-27.
[34] Bryan MB, Scott AP, Li W. Sex steroids and their receptors in lampreys. Steroids. 2008;73:1-12.
[35] Rafestin-Oblin ME, Souque A, Bocchi B, Pinon G, Fagart J, Vandewalle A. The severe form of hypertension caused by the activating S810L mutation in the mineralocorticoid receptor is cortisone related. Endocrinology. 2003;144:528-33.
[36] Bridgham JT, Ortlund EA, Thornton JW. An epistatic ratchet constrains the direction of glucocorticoid receptor evolution. Nature. 2009;461:515-9.