Origin and diversification of steroids: Co-evolution of enzymes and nuclear receptors

Michael E. Baker
Department of Medicine, 0693
University of California, San Diego
9500 Gilman Drive
La Jolla, California 92093-0693

E-mail: mbaker@ucsd.edu
Phone: 858-534-8317
Fax: 858-822-0873

Summary Recent sequencing of amphioxus and sea urchin genomes has provided important data for understanding the origins of enzymes that synthesize adrenal and sex steroids and the receptors that mediate physiological response to these vertebrate steroids. Phylogenetic analyses reveal that CYP11A and CYP19, which are involved in the synthesis of adrenal and sex steroids, first appear in the common ancestor of amphioxus and vertebrates. This correlates with recent evidence for the first appearance in amphioxus of receptors with close similarity to vertebrate steroid receptors. Other CYP450 enzymes involved in steroid synthesis can be traced back to invertebrates, in which they have at least two functions: detoxifying xenobiotics and catalyzing the synthesis of sterols that activate nuclear receptors. CYP450 metabolism of hydrophobic xenobiotics may have been a key event in the origin of ligand-activated steroid receptors from constitutively active nuclear receptors.

Introduction

This review of the origin and diversification of steroids focuses on adrenal and sex steroids - aldosterone, cortisol, estradiol (E2), progesterone and testosterone -, which regulate a wide range of physiological processes including reproduction, development and homeostasis [Figure 1]. Because the physiological actions of these vertebrate steroids are mediated by nuclear receptors, a large and diverse group of transcription factors that arose in multicellular animals [1-3], the origin and diversification of steroids involves the co-evolution of enzymes that synthesize steroids from cholesterol [4-5] [Figure 2] and the receptors that mediate their

responses [1-2,6-7]. In this manuscript, steroids and steroid receptors refer to vertebrate steroids and their nuclear receptors, which are different from ecdysteroids and the ecdysone receptor [EcR] [2,8-9].

Important in the orgins and evolution of vertebrate steroids are two important classes of enzymes, cytochrome P450s [CYP450s] [10-12] and hydroxysteroid dehydrogenases [HSDs] [13-15], which are involved in the synthesis of biologically active steroids from cholesterol. CYP450s are a large and ancient enzyme family that has an important role in detoxification of chemicals in invertebrates as well as vertebrates [1,7,10-12]. As will be discussed later, this detoxification function may have been important in the co-evolution of steroids and nuclear receptors [1,7].

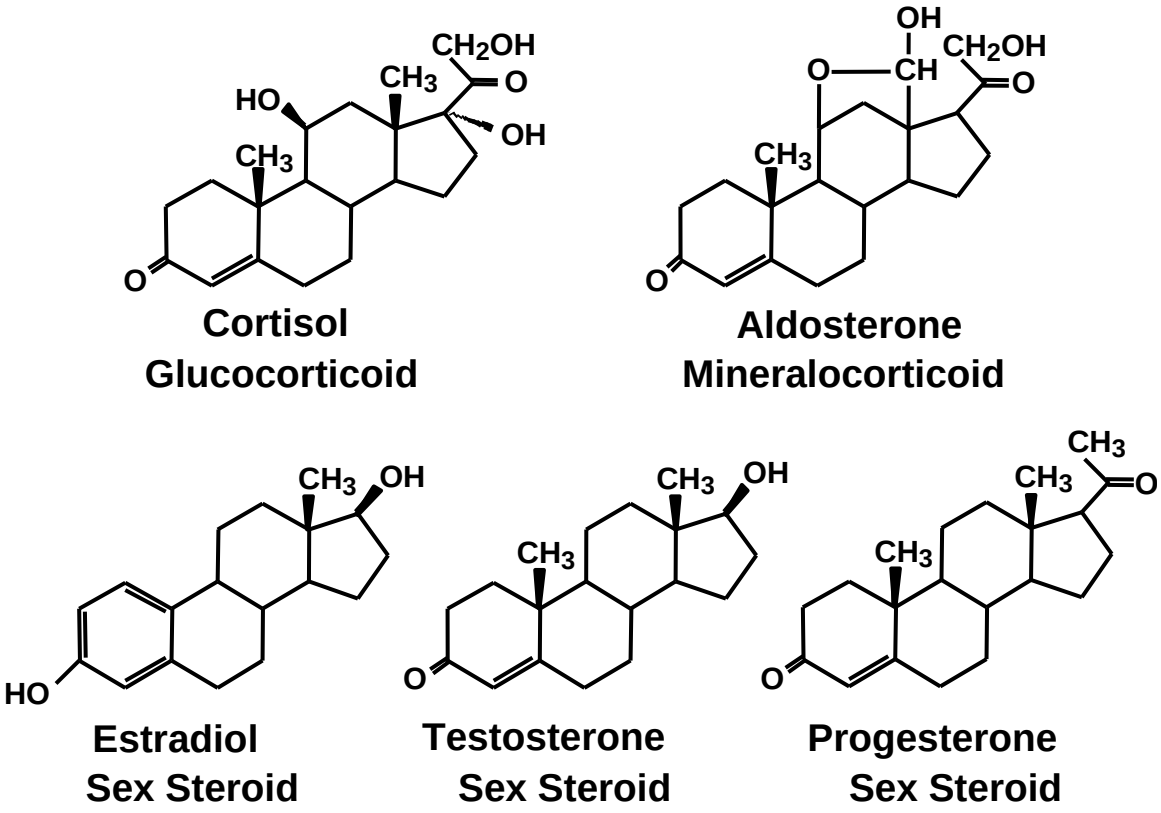


Figure 1. Structures of adrenal and sex steroids.

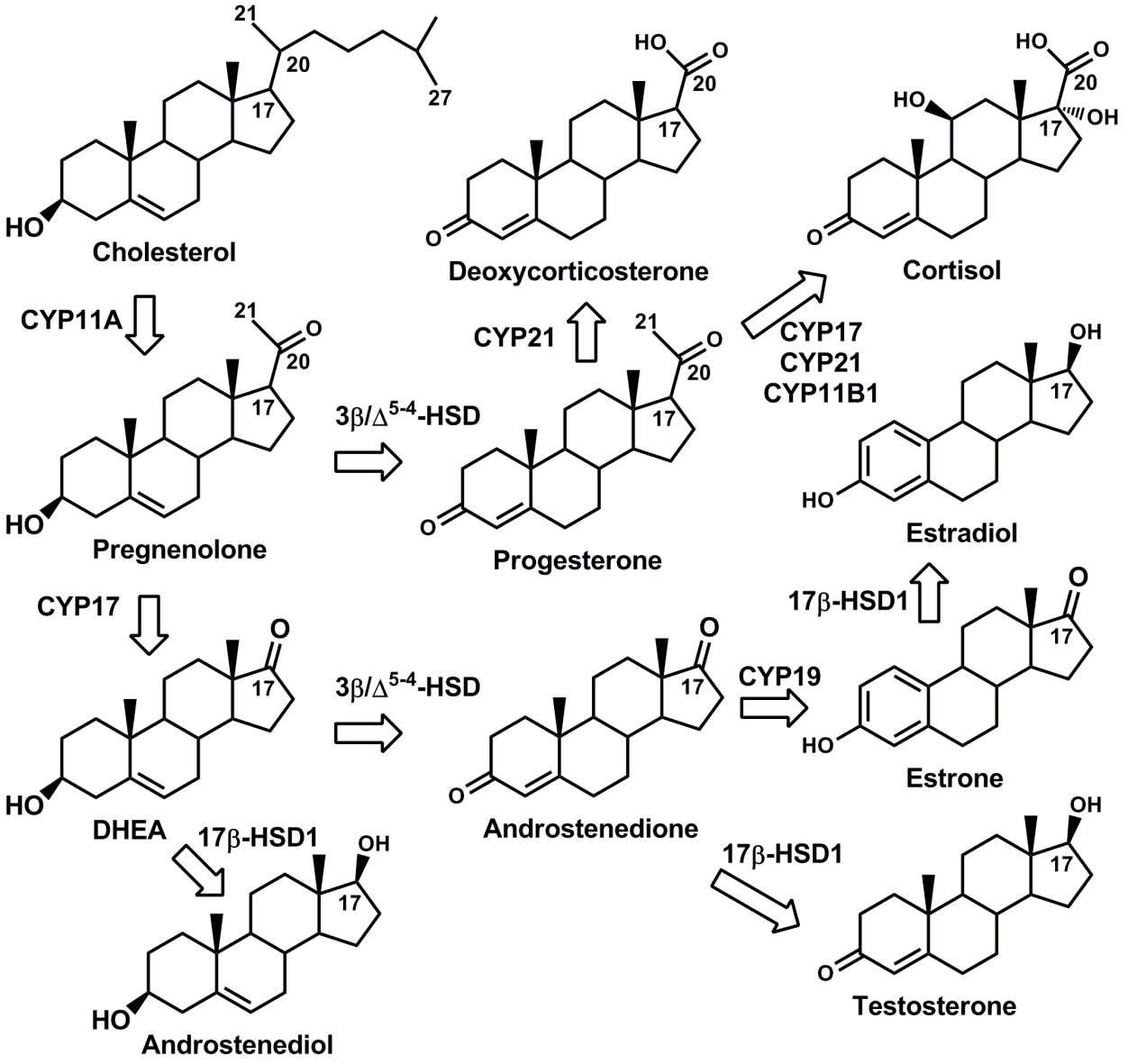


Figure 2. Enzymes involved in the synthesis of vertebrate steroids from cholesterol

CYP450s, 3β/Δ^{5-4} HSD and 17β-HSD-type 2 catalyze the formation of vertebrate steroids from cholesterol [4-5,7,10-12]. CYP11A and CYP19 are present in amphioxus [7,37,40,63]. Δ^5 steroids, such as Δ^5-androstenediol, may be ancestral ligands for the ER [6,42]. Similarly, deoxycorticosterone may be the ancestral mineralocorticoid [64-65]

To understand the evolution of steroids it is important to consider when nuclear receptors, in general, and adrenal and sex steroid receptors in particular, evolved, and how this correlates with the evolution of enzymes involved in steroid synthesis. We begin with the origins of nuclear receptors.

Nuclear receptors are unique to multicellular animals.

The availability of complete genomes from bacteria, yeast, plants, invertebrates and vertebrates has increased our understanding of the origins of various transcription factors, including nuclear receptors. Searches of Genbank did not find any nuclear receptors in the yeast *Saccharomyces cerevisiae* [16] or in the plant *Arabidopsis* [17]. This was surprising because nuclear receptors, such as the estrogen receptor (ER) [18] and glucocorticoid receptor (GR) [19], can function nicely when transfected into yeast along with a reporter gene. Similarly, the glucocorticoid receptor can function when transfected into *Arabidopsis* [20]. This indicates that the basic machinery for transcriptional activation by nuclear receptors evolved in the common ancestor of fungi and metazoans, even if this ancestor did not contain nuclear receptors.

In fact, nuclear receptors are found in sponges and simple multicellular animals [21-23]. The "sudden" appearance of nuclear receptors in basal multicellular animals, suggests that nuclear receptors had an important role in this important evolutionary transition.

When did steroid receptors evolve?

The origins of steroid receptors have been a subject of much interest [24-27]. In 1997, two different analyses suggested that steroid receptors arose in deuterostomes [Figure 3]. Escriva et al [26] used PCR to investigate the presence of steroid and thyroid hormone receptors and other nuclear receptors in various deuterostomes and protostomes. They found evidence for steroid receptors in hagfishes, but not in sea urchin or acorn worm, which are at the base of the deuterostome line. Nor did they find an ortholog of an adrenal or sex steroid receptor in a protostome. This suggested that steroid binding arose late in metazoan evolution in a primitive deuterostome.

A similar conclusion came from an alternative approach using a phylogenetic analysis of the ligand-binding domains of receptors for steroids, thyroid hormone, retinoids, vitamin D and ecdysone [24]. This phylogenetic analysis indicated that steroid receptors arose at the base of the vertebrate line, in an ancestral cephalochordate [e.g. amphioxus] or urochordate [e.g. Ciona]. It predicted that orthologs of adrenal and sex steroid receptors would not be found in the genomes of either *Caenorhabditis elegans* or *Drosophila*, which were still being sequenced. This analysis also proposed that the ER was the ancestral steroid receptor, and that a duplication

of this ER followed by sequence divergence and further duplications led to the androgen receptor (AR), GR, mineralocorticoid receptor (MR) and progesterone receptor (PR). It also suggested

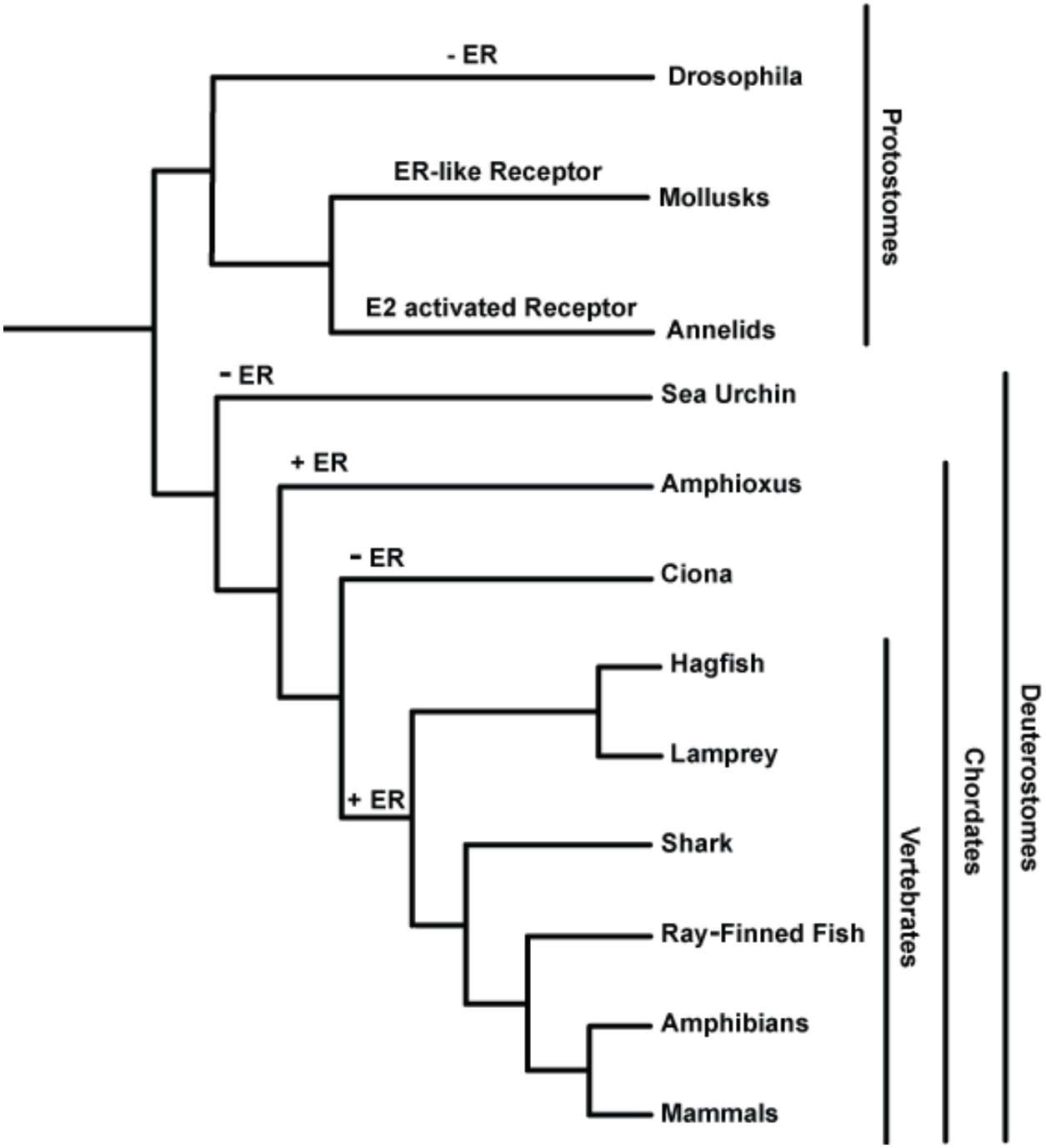


Figure 3. Estrogen-binding nuclear receptors in metazoans

Amphioxus contains an ortholog of vertebrate ERs. Annelids contain nuclear receptors that are activated by estradiol [49]. Nuclear receptors with sequence similarity to vertebrate ERs are found in mollusks, and these receptors do not bind estradiol [43-45,47-48]. Amphioxus also contains an SR, which is an ancestor of 3keto-steroid receptors found in vertebrates [not shown].

that the ancestral ER had functions that differed from its well studied reproductive functions in mammals [28]. Indeed, since the transition from amphioxus to lamprey involves the evolution of the head [29-32] [Figure 4], it seemed reasonable to propose a role for the ER in the evolution of a more complex brain in vertebrates [24]. As more nuclear receptor sequences became available, the phylogenetic evidence for the ER as the ancestral steroid receptor became stronger [25]. It was the cloning of the lamprey ER, PR and corticoid receptors by Thornton [27] that firmly established that the ER was the ancestral steroid receptor.

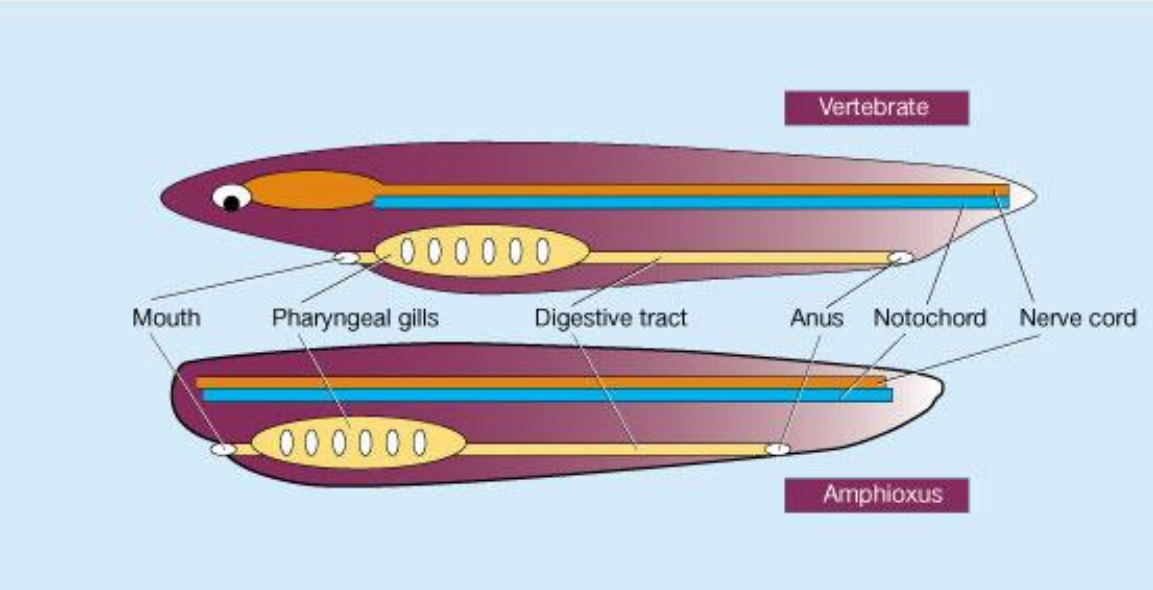


Figure 4. Evolution of the head in amphioxus [29-32]
The notochord extends right to the front of amphioxus. In contrast, the vertebrate has a prominent head at the anterior end, extending beyond the notochord. **Modified from [29].**

A role for steroid receptors in vertebrate evolution?

In 2001, the human genome was sequenced and found to have about 33,000 genes, which were many fewer than previously thought, and not much more than the ~18,000 genes in *C. elegans* and ~13,000 genes in *Drosophila*. Since then, these genomes have been analyzed further and the human, *C. elegans* and *Drosophila* genomes are now estimated to contain ~23,500, ~20,000 and ~14,000 genes, respectively. These revisions make the low number of genes in the human genome relative to *C. elegans* and *Drosophila* even more perplexing. Several explanations have been advanced to explain the relatively small difference between the

number of genes in the genomes of humans, worms and flies including alternative splicing, the presence of multiple domains in vertebrate proteins, which increases the complexity of protein-protein interactions, post-translational modifications of vertebrate proteins and the evolution of networks of transcription factors [see [28] for original references].

Another explanation that was proposed for complex differentiation and development in vertebrates was the emergence of one or more steroid receptors in amphioxus, a basal chordate [28]. Subsequent expansion and diversification of these receptors as a result of two genome size duplications, which occurred between the evolution of amphioxus and agnathostomes [e.g. lamprey, hagfish] [33], yielded the adrenal and sex steroid receptors [28]. This hypothesis that steroid receptors arose in amphioxus and expanded and diversified during the interval leading to basal vertebrates was based on searches of GenBank that did not find invertebrate ancestors of vertebrate steroid receptors or of steroid dehydrogenases [7,13,15,34]. If the suite of steroid receptors and steroid dehydrogenases that are essential for the adrenal and sex steroid response arose and diversified at the base of the vertebrate line, then it would provide an additional means for regulating differentiation, development and homeostasis, and contribute to the evolution of complex regulatory networks [e.g. nervous and immune systems] found in vertebrates.

Amphioxus contains steroid receptors

Recent studies support the presence of steroid signaling in amphioxus (*Branchiostoma floridae*) and *B. belcheri* [35-39]. Key enzymes for the synthesis of testosterone and estradiol are present in amphioxus [37,40]. Moreover, there is an ER in *B. floridae* (BfER) [36,38] and *B. belcheri* (BbER) [39] and steroid receptors (BfSR and BbSR), which are phylogenetically closest to 3keto-steroid receptors[36,39]. Unexpectedly, BfER and BbER are not activated by estradiol or other steroids [36,38-39]. In contrast, BfSR and BbSR activate transcription in the presence of E2 and estrone (E1), but not in the presence of 3keto-steroids [36,39]. Interestingly, BfER and BbER repress activation of BfSR and BbSR, respectively, by E2, suggesting a novel cross-regulatory interaction between these two receptors. The affinity of E2 for BfSR is about 100 nM [36], which makes it unlikely that E2 is the physiological ligand [41]. Other steroids may be the physiological ligands [6,35,42]

Invertebrate ERs in mollusks.

The origins steroid receptors became more complex with the cloning of a nuclear receptor that had strong sequence similarity to the human ER from *Aplysia californica* [43]. The sequence of the DNA-binding domain of *Aplysia* ER is 79% and 65% identical, respectively,

to the DNA-binding domain on human ER and ERR. This indicates a deep conservation of the interaction of between the hormone response element and the DNA-binding domains on human ER, human ERR and Aplysia ER.

In contrast, the ligand-binding domain of *Aplysia* ER is about 35% identical to human ER after insertion of gaps at 17 positions in the alignment, which indicates substantial divergence in their ligand-binding domains. Interestingly, Aplysia ER is about 32% identical to human ERR with gaps at 5 positions. Analysis of the ligand-binding domain on octopus ER, another invertebrate ER [44], reveals that it is 33% identical to human ERα, after insertion of gaps at 21 positions in the alignment. Octopus ER is 29% identical to human ERR, after insertion of gaps at 5 positions. An explanation for the fewer gaps in the alignments of the ligand-binding domain on octopus ER and *Aplysis* ER with human ERR is that their lengths are shorter than the ligand-binding domain of human ERα [45-46]. . Aplysia ER and octopus ER do not bind E2 or other steroids. In fact, the *Aplysia* ER and octopus ER constitutively regulate gene transcription in the absence of E2 or other steroids. In both of these biological properties, *Aplysia* ER and octopus ER are similar to human ERR [46]. As happens with intriguing discoveries, soon other invertebrate receptors with sequence similarity to vertebrate ERs were cloned from snails [47] andscallops [48]. These invertebrate receptors also were constitutively active and did not bind E2 or other steroids.

An insight into the basis for the absence of E2 binding to octopus ER came from a 3D model of octopus ER complexed with E2, which revealed that the ligand-binding site on octopus ER was too small to contain E2 [45]. As a result, there were steric clashes between E2 and side chains in octopus ER, which prevented E2 from occupying octopus ER, providing an explanation for the absence of steroid binding. The small ligand-binding pocket in octopus ER is consistent with is shorter sequence [45-46]. Similar to the 3D model of octopus ER with E2, Greschik et al [46] showed that in human ERRγ the ligand binding site is occupied by side chains from neighboring amino acids, which prevent binding of E2.

Annelid nuclear receptors bind estradiol

Recently, nuclear receptors from two annelids, *Platynereis dumerilii* and *Capitella capitata*, were found to regulate gene transcription in the presence of E2 [49]. Measurements of transcription in cells transfected with the *P. dumerilii* receptor yielded an EC50 of 8.5 nM for E2. In the presence of 1 μM E2, the *P. dumerilii* receptor increased transcription by about 3.5-fold over controls. At 10 nM E2, transcription increased by about 2-fold, which does not

appear to be physiological by mammalian standards for E2 activation of ERα and is substantially lower than the ~35-fold increase in activity for human ERα in the presence of 10 nM E2 [49]. Various *in vivo* conditions may lead to the *P. dumerilii* ER having a stronger response to E2 at lower concentrations: It may be that there are specific coactivators in *P. dumerilii* that are necessary for optimal transcriptional activity of its receptor in the presence of E2; Or perhaps there are post-translational modifications of this receptor in *P. dumerilii* that increase its response to E2; Or physiological activity of the *P. dumerilii* receptor may require the DNA-binding sequence in *P. dumerilii* [50]. Another explanation is that E2 may not be the physiological ligand for the *P. dumerilii* receptor. Deciphering the ligands and the biological function(s) of the *P. dumerilii* receptor for E2 will yield important information about ligand-activated nuclear receptors in annelids, which are still poorly understood.

Divergent Evolution, Convergent Evolution, Horizontal Transfer?

The presence of estrogen receptor-like nuclear receptors in mollusks and annelids leaves the origins of steroid receptor signaling unsettled. There are at least three possible explanations for these data. One explanation is that the mollusk, annelid and vertebrate receptors are an example of divergent evolution from an ancestral steroid receptor, which evolved in a common ancestor of protostomes and deuterostomes [43,49]. Subsequently, in some mollusks, mutations in this steroid receptor resulted in the loss of E2 binding and the evolution of constitutive activity. In other organisms, such as flies and worms, the ancestral ER gene was lost.

A second explanation, which we favor, is that the similarities between invertebrate ERs and vertebrate ERs are an example of convergent evolution from an ancestral ERR [2,21]. As noted previously, convergent evolution is common in steroid binding proteins [13]. Indeed, high affinity binding for E2 is found in vertebrate ERs, rat and mouse alpha-fetoprotein and sex steroid binding globulin [13], as well as in enzymes in yeast [51], which do not have steroid receptors. Another example of convergence is the presence of over ten 17β-hydroxysteroid dehydrogenases that metabolize estrogens and androgens [13-14,52].

This explanation is consistent with the similarities in the structural properties of octopus ER and human ERR [45], which support the closeness of octopus ER to human ERR. Similarly, binding of E2 by receptors in *P. dumerilii* and *C. capitata* may have evolved through convergent evolution from an ancestral ERR. Interestingly, conversion of human ERRγ to bind E2 has been accomplished by Greschik et al. [46] by mutation of two residues in the ligand binding

pocket. However, this mutant ERRγ remained constitutively active. Understanding the relationship of the structure of the *P. dumerilii* ER-like receptor to ERRs and vertebrate ERs should elucidate steps in evolution of ligand-activated receptors from constitutive receptors.

A third explanation is that there was horizontal transfer between a protostome and deuterostome. Horizontal transfer is common in prokaryotes [53] and rare in eukaryotes, especially multi-cellular animals. Nevertheless this possibility must be considered.

It is clear that at this time that there are unresolved questions about the evolution of steroid receptors in metazoans. Thus, there is a need for alternative methods to investigate the origins of steroid hormone signaling.

Origin of key steroidogenic enzymes in amphioxus

Fortunately, an examination of the origins of steroidogenic enzymes [1,7,28] provides an alternate approach to understanding the origins of steroid hormone signaling. When did the enzymes that synthesize E2 and other vertebrate steroids evolve? The evolution of CYP11A, which catalyzes the cleavage of the cholesterol side chain to form pregnenolone, and CYP19, which catalyzes the formation of estradiol from testosterone [Figure 2] [5,11] provide important clues to the origin of signaling by vertebrate steroids. Orthologs of both enzymes have been cloned from amphioxus [37,40]. A recent extensive search of GenBank by Markov et al [7] did not find evidence for a CYP11A or CYP19 in species other than amphioxus or vertebrates, suggesting that these enzymes arose in the ancestor of chordates.

Further support comes from database searches for orthologs of 17β-HSD-type 1 and 17β-HSD -type 2, which are involved in estrogen synthesis [14-15,25]. There are many 17β-HSD homologs in invertebrates, for which the biological substrate has not been determined [14]. However, at this time, orthologs of 17β-HSD-type 1 and 17β-HSD -type 2 have been found only in vertebrates [7].

These recent database searches indicate that some of the key enzymes involved in the synthesis of adrenal and sex steroids first evolved in amphioxus, when the ligand-activated BfSR and BbSR first appear. Of course, as noted by Markov et al [7], there may have been convergent evolution for aromatase activity in invertebrates. Similarly, convergent evolution could have led to CYP11A, 17β-HSD1 and 17β-HSD2 activities in invertebrates.

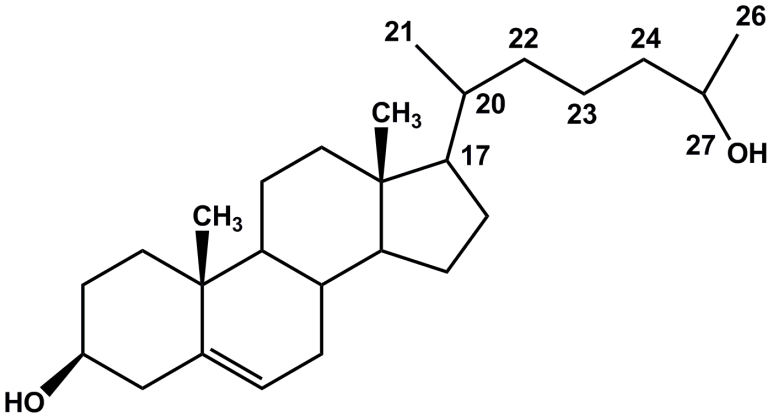
These studies raise the question of how vertebrate steroids and their receptors evolved from an ancestral nuclear receptor, which we discuss next.

Role of CYP450 metabolism of sterols and xenobiotics in the ancestry of steroidogenic enzymes

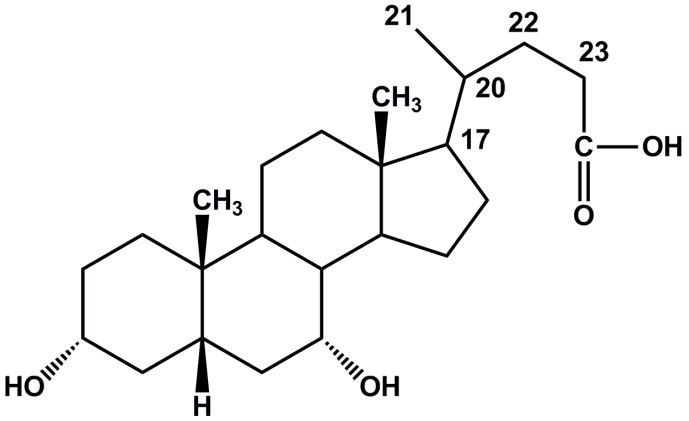
CYP450s are ancient enzymes, which are found in bacteria, yeast and basal metazoans [7,10-12]. CYP450s evolved through gene duplication and divergence into a diverse protein family that metabolizes a wide variety of chemicals. Two functions of ancient CYP450s provide clues to the evolution of vertebrate steroids. First, CYP450s catalyze the synthesis of sterols, such as oxysterols and bile acids from cholesterol [Figure 3]. Sterols and bile acids regulate gene transcription by binding to nuclear receptors [1,7-8,54-55]. Oxysterols are ligands for the liver X receptor [LXR], which acts as an oxysterol sensor [54-55], and bile acids are ligands for the farnesoid X receptor [FXR], vitamin D receptor [VDR] and pregnane X receptor [PXR] [54-55]. In insects and crustaceans, CYP450s also catalyze the synthesis of 20-hydroxyecdysone from cholesterol. 20-hydroxyecdysone regulates development and reproduction in arthropods through binding to the ecdysone receptor, which belongs to the nuclear receptor family [2,8].

A second function of invertebrate and vertebrate CYP450s is to hydroxylate a wide variety of xenobiotics, as part of the process for removing toxic chemicals. A recent phylogenetic analysis found that some CYP450s that synthesize steroids [Figure 2] evolved from CYP450s involved in detoxification of xenobiotics [7].

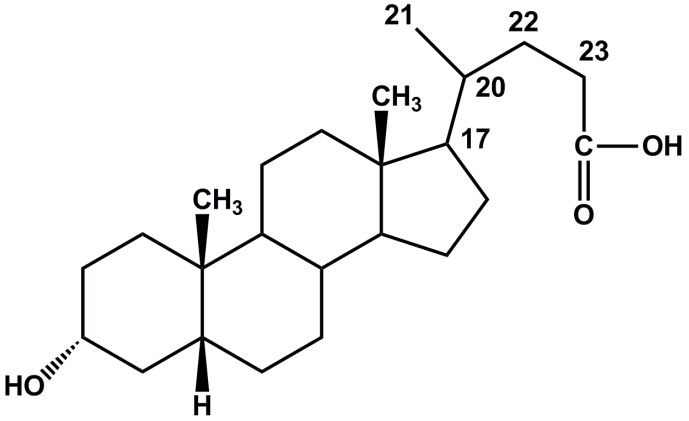
At this time, the identity of the ligand(s) that activated the ancestral ER is not known. The vertebrate ER is notable for binding of environmental chemicals with diverse structures [56-57], the ancestral ER may have been a xenobiotic sensor [7]. The binding of many xenobiotics and natural products to the ER is consistent with recent evidence that the vertebrate ER has conformational flexibility, which allows it to bind diverse ligands, including 27-hydroxycholesterol [58-59] [Figure 5], trifluoromethyl-phenylvinyl-E2 [60], as well as tamoxifen and diethylstilbestrol. Thus, there may have been several classes of ligands, including sterols, xenobiotics and natural chemicals that were the original ligand(s) for the ancestral ER.



27-hydroxy-Cholesterol



Chenodeoxycholic Acid



Lithocholic Acid

FIG.5. Structures of 27-hydroxycholesterol and bile acids.

Cholesterol is a precursor for oxysterols and bile acids, both of which bind to nuclear receptors and regulate gene transcription [54-55]. These ligands include 27-hydroxy-cholesterol, an oxysterol, andcholic acid, and lithocholic acid, which are bile acids.. Oxysterols are ligands for the liver X receptor [LXR] [54-55]; bile acids are ligands for the farnesoid X receptor [FXR], vitamin D receptor [VDR] and pregnane X receptor [PXR] [54-55].

In summary, searches of recently completed deuterostome and protostome genomes for orthologs of vertebrate steroid receptors and steroidogenic enzymes support an earlier hypothesis that the ligand-activated ER and other vertebrate steroid receptors evolved in a deuterostome [3,7,25-26]. The close sequence and structural similarity of the invertebrate ERs and ERR [46] and the presence of ERRs in primitive metazoans [2,21] suggests that the ancestral invertebrate ER evolved from an ERR and the ER ancestors was likely to have been constitutively active. This leaves unresolved how ligand activation of the vertebrate ER evolved from a constitutively active ancestor, which appears to be a complex process because a mutant human ERR that binds E2 [46] and a mutant *Drosophila* ERR [61] that binds diethylstilbestrol have been constructed and neither activates transcription with the bound ligand. The mutant human ERR complexed with E2 is constitutively active [46]. Interestingly, binding of diethylstilbestrol to the mutant *Drosphila* ERR represses constitutive activity [61]. Further complicating the relationship between binding of E2 to the ER and transcriptional activity is the evidence that, point mutations in ERα lead to a constitutively active receptor [62]. Sorting out these latter steps in evolution of a ligand-activated ER is an important next task in the deciphering the evolution of adrenal and sex steroid action.

Acknowledgments

Many of my ideas on the evolution of steroids and their receptors were presented at the 1999 and 2009 E.hormone meetings at Tulane University. I thank the participants for their questions and comments just after my talk and later during informal discussions at the Gravier Street Salon. I also thank G. Markov and V. Laudet for helping me to crystallize my ideas.

References

[1] Baker, M.E. (2005). Xenobiotics and the evolution of multicellular animals: emergence and diversification of ligand-activated transcription factors. Integrative and Comparative Biology 45, 172-178.

[2] Bertrand, S., Brunet, F.G., Escriva, H., Parmentier, G., Laudet, V. and Robinson-Rechavi, M. (2004). Evolutionary genomics of nuclear receptors: from twenty-five ancestral genes to derived endocrine systems. Mol Biol Evol 21, 1923-37.

[3] Escriva, H., Delaunay, F. and Laudet, V. (2000). Ligand binding and nuclear receptor evolution. Bioessays 22, 717-27.

[4] Nebert, D.W. and Russell, D.W. (2002). Clinical importance of the cytochromes P450. Lancet 360, 1155-62.

[5] Payne, A.H. and Hales, D.B. (2004). Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. Endocr Rev 25, 947-70.

[6] Baker, M.E. (2004). Co-evolution of steroidogenic and steroid-inactivating enzymes and adrenal and sex steroid receptors. Mol Cell Endocrinol 215, 55-62.

[7] Markov, G.V., Tavares, R., Dauphin-Villemant, C., Demeneix, B.A., Baker, M.E. and Laudet, V. (2009). Independent elaboration of steroid hormone signaling pathways in metazoans. Proc Natl Acad Sci U S A 106, 11913-8.

[8] Rewitz, K.F. and Gilbert, L.I. (2008). Daphnia Halloween genes that encode cytochrome P450s mediating the synthesis of the arthropod molting hormone: evolutionary implications. BMC Evol Biol 8, 60.

[9] Spindler, K.D., Honl, C., Tremmel, C., Braun, S., Ruff, H. and Spindler-Barth, M. (2009). Ecdysteroid hormone action. Cell Mol Life Sci 66, 3837-50.

[10] Danielson, P.B. (2002). The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. Curr Drug Metab 3, 561-97.

[11] Nelson, D.R. (2003). Comparison of P450s from human and fugu: 420 million years of vertebrate P450 evolution. Arch Biochem Biophys 409, 18-24.

[12] Thomas, J.H. (2007). Rapid birth-death evolution specific to xenobiotic cytochrome P450 genes in vertebrates. PLoS Genet 3, e67.

[13] Baker, M.E. (2001). Evolution of 17beta-hydroxysteroid dehydrogenases and their role in androgen, estrogen and retinoid action. Mol Cell Endocrinol 171, 211-5.

[14] Moeller, G. and Adamski, J. (2009). Integrated view on 17beta-hydroxysteroid dehydrogenases. Mol Cell Endocrinol 301, 7-19.

[15] Peltoketo, H., Luu-The, V., Simard, J. and Adamski, J. (1999). 17beta-hydroxysteroid dehydrogenase (HSD)/17-ketosteroid reductase (KSR) family; nomenclature and main characteristics of the 17HSD/KSR enzymes. J Mol Endocrinol 23, 1-11.

[16] Goffeau, A. et al. (1996). Life with 6000 genes. Science 274, 546, 563-7.

[17] Arabidopsis, Genome and Initiative. (2000). Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. Nature 408, 796-815.

[18] Metzger, D., White, J.H. and Chambon, P. (1988). The human oestrogen receptor functions in yeast. Nature 334, 31-6.

[19] Schena, M. and Yamamoto, K.R. (1988). Mammalian glucocorticoid receptor derivatives enhance transcription in yeast. Science 241, 965-7.

[20] Schena, M., Lloyd, A.M. and Davis, R.W. (1991). A steroid-inducible gene expression system for plant cells. Proc Natl Acad Sci U S A 88, 10421-5.

[21] Baker, M.E. (2008). Trichoplax, the simplest known animal, contains an estrogen-related receptor but no estrogen receptor: Implications for estrogen receptor evolution. Biochem Biophys Res Commun 375, 623-7.

[22] Reitzel, A.M. and Tarrant, A.M. (2009). Nuclear receptor complement of the cnidarian Nematostella vectensis: phylogenetic relationships and developmental expression patterns. BMC Evol Biol 9, 230.

[23] Wiens, M., Batel, R., Korzhev, M. and Muller, W.E. (2003). Retinoid X receptor and retinoic acid response in the marine sponge Suberites domuncula. J Exp Biol 206, 3261-71.

[24] Baker, M.E. (1997). Steroid receptor phylogeny and vertebrate origins. Mol Cell Endocrinol 135, 101-7.

[25] Baker, M.E. (2001). Adrenal and sex steroid receptor evolution: environmental implications. J Mol Endocrinol 26, 119-25.

[26] Escriva, H. et al. (1997). Ligand binding was acquired during evolution of nuclear receptors. Proc Natl Acad Sci U S A 94, 6803-8.

[27] Thornton, J.W. (2001). Evolution of vertebrate steroid receptors from an ancestral estrogen receptor by ligand exploitation and serial genome expansions. Proc Natl Acad Sci U S A 98, 5671-6.

[28] Baker, M.E. (2003). Evolution of adrenal and sex steroid action in vertebrates: a ligand-based mechanism for complexity. Bioessays 25, 396-400.

[29] Finnerty, J.R. (2000). Evolutionary developmental biology. Head start. Nature 408, 778-9, 781.

[30] Schubert, M., Escriva, H., Xavier-Neto, J. and Laudet, V. (2006). Amphioxus and tunicates as evolutionary model systems. Trends Ecol Evol 21, 269-77.

[31] Bronner-Fraser, M. (2008). On the trail of the 'new head' in Les Treilles. Development 135, 2995-9.

[32] Garcia-Fernandez, J. and Benito-Gutierrez, E. (2009). It's a long way from amphioxus: descendants of the earliest chordate. Bioessays 31, 665-75.

[33] Holland, P.W., Garcia-Fernandez, J., Williams, N.A. and Sidow, A. (1994). Gene duplications and the origins of vertebrate development. Dev Suppl, 125-33.

[34] Edwards, C.R., Benediktsson, R., Lindsay, R.S. and Seckl, J.R. (1996). 11 beta-Hydroxysteroid dehydrogenases: key enzymes in determining tissue-specific glucocorticoid effects. Steroids 61, 263-9.

[35] Baker, M.E. (2007). Amphioxus, a primitive chordate, is on steroids: evidence for sex steroids and steroidogenic enzymes. Endocrinology 148, 3551-3.

[36] Bridgham, J.T., Brown, J.E., Rodriguez-Mari, A., Catchen, J.M. and Thornton, J.W. (2008). Evolution of a new function by degenerative mutation in cephalochordate steroid receptors. PLoS Genet 4, e1000191.

[37] Mizuta, T. and Kubokawa, K. (2007). Presence of sex steroids and cytochrome P450 genes in amphioxus. Endocrinology 148, 3554-65.

[38] Paris, M., Pettersson, K., Schubert, M., Bertrand, S., Pongratz, I., Escriva, H. and Laudet, V. (2008). An amphioxus orthologue of the estrogen receptor that does not bind estradiol: insights into estrogen receptor evolution. BMC Evol Biol 8, 219.

[39] Katsu, Y., Kubokawa, K., Urushitani, H. and Iguchi, T. (2010). Estrogen-dependent transactivation of amphioxus steroid hormone receptor via both estrogen and androgen response elements. Endocrinology 151, 639-48.

[40] Castro, L.F., Santos, M.M. and Reis-Henriques, M.A. (2005). The genomic environment around the Aromatase gene: evolutionary insights. BMC Evol Biol 5, 43.

[41] Baker, M.E. and Chang, D.J. (2009). 3D model of amphioxus steroid receptor complexed with estradiol. Biochem Biophys Res Commun 386, 516-20.

[42] Baker, M.E. (2002). Recent insights into the origins of adrenal and sex steroid receptors. J Mol Endocrinol 28, 149-52.

[43] Thornton, J.W., Need, E. and Crews, D. (2003). Resurrecting the ancestral steroid receptor: ancient origin of estrogen signaling. Science 301, 1714-7.

[44] Keay, J., Bridgham, J.T. and Thornton, J.W. (2006). The Octopus vulgaris estrogen receptor is a constitutive transcriptional activator: evolutionary and functional implications. Endocrinology 147, 3861-9.

[45] Baker, M.E. and Chandsawangbhuwana, C. (2007). Analysis of 3D models of octopus estrogen receptor with estradiol: evidence for steric clashes that prevent estrogen binding. Biochem Biophys Res Commun 361, 782-8.

[46] Greschik, H., Wurtz, J.M., Sanglier, S., Bourguet, W., van Dorsselaer, A., Moras, D. and Renaud, J.P. (2002). Structural and functional evidence for ligand-independent transcriptional activation by the estrogen-related receptor 3. Mol Cell 9, 303-13.

[47] Bannister, R., Beresford, N., May, D., Routledge, E.J., Jobling, S. and Rand-Weaver, M. (2007). Novel estrogen receptor-related Transcripts in Marisa cornuarietis; a freshwater snail with reported sensitivity to estrogenic chemicals. Environ Sci Technol 41, 2643-50.

[48] Matsumoto, T., Nakamura, A.M., Mori, K., Akiyama, I., Hirose, H. and Takahashi, Y. (2007). Oyster estrogen receptor: cDNA cloning and immunolocalization. Gen Comp Endocrinol 151, 195-201.

[49] Keay, J. and Thornton, J.W. (2009). Hormone-activated estrogen receptors in annelid invertebrates: implications for evolution and endocrine disruption. Endocrinology 150, 1731-8.

[50] Meijsing, S.H., Pufall, M.A., So, A.Y., Bates, D.L., Chen, L. and Yamamoto, K.R. (2009). DNA binding site sequence directs glucocorticoid receptor structure and activity. Science 324, 407-10.

[51] Malloy, P.J., Zhao, X., Madani, N.D. and Feldman, D. (1993). Cloning and expression of the gene from Candida albicans that encodes a high-affinity corticosteroid-binding protein. Proc Natl Acad Sci U S A 90, 1902-6.

[52] Desnoyers, S., Blanchard, P.G., St-Laurent, J.F., Gagnon, S.N., Baillie, D.L. and Luu-The, V. (2007). Caenorhabditis elegans LET-767 is able to metabolize androgens and estrogens and likely shares common ancestor with human types 3 and 12 17beta-hydroxysteroid dehydrogenases. J Endocrinol 195, 271-9.

[53] Doolittle, W.F. and Bapteste, E. (2007). Pattern pluralism and the Tree of Life hypothesis. Proc Natl Acad Sci U S A 104, 2043-9.

[54] Chawla, A., Repa, J.J., Evans, R.M. and Mangelsdorf, D.J. (2001). Nuclear receptors and lipid physiology: opening the X-files. Science 294, 1866-70.

[55] Hylemon, P.B., Zhou, H., Pandak, W.M., Ren, S., Gil, G. and Dent, P. (2009). Bile acids as regulatory molecules. J Lipid Res 50, 1509-20.

[56] Baker, M.E. (2002). Albumin, steroid hormones and the origin of vertebrates. J Endocrinol 175, 121-7.

[57] Diamanti-Kandarakis, E., Bourguignon, J.P., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., Zoeller, R.T. and Gore, A.C. (2009). Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr Rev 30, 293-342.

[58] DuSell, C.D., Umetani, M., Shaul, P.W., Mangelsdorf, D.J. and McDonnell, D.P. (2008). 27-hydroxycholesterol is an endogenous selective estrogen receptor modulator. Mol Endocrinol 22, 65-77.

[59] Umetani, M. et al. (2007). 27-Hydroxycholesterol is an endogenous SERM that inhibits the cardiovascular effects of estrogen. Nat Med 13, 1185-92.

[60] Nettles, K.W. et al. (2007). Structural plasticity in the oestrogen receptor ligand-binding domain. EMBO Rep 8, 563-8.

[61] Ostberg, T., Jacobsson, M., Attersand, A., Mata de Urquiza, A. and Jendeberg, L. (2003). A triple mutant of the Drosophila ERR confers ligand-induced suppression of activity. Biochemistry 42, 6427-35.

[62] Lazennec, G., Ediger, T.R., Petz, L.N., Nardulli, A.M. and Katzenellenbogen, B.S. (1997). Mechanistic aspects of estrogen receptor activation probed with constitutively active estrogen receptors: correlations with DNA and coregulator interactions and receptor conformational changes. Mol Endocrinol 11, 1375-86.

[63] Reitzel, A.M. and Tarrant, A.M. (2010). Correlated evolution of androgen receptor and aromatase revisited. Mol Biol Evol

[64] Baker, M.E. (2003). Evolution of glucocorticoid and mineralocorticoid responses: go fish. Endocrinology 144, 4223-5.

[65] Baker, M.E., Chandsawangbhuwana, C. and Ollikainen, N. (2007). Structural analysis of the evolution of steroid specificity in the mineralocorticoid and glucocorticoid receptors. BMC Evol Biol 7, 24.