11β-hydroxysteroid dehydrogenase-type 2 evolved from an ancestral 17β-hydroxysteroid dehydrogenase-type 2

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Abstract. 11β-hydroxysteroid dehydrogenase type-2 (11β-HSD2) regulates the local concentration of cortisol that can activate the glucocorticoid receptor and mineralocorticoid receptor, as well as the concentration of 11-keto-testosterone, the active androgen in fish. Similarly, 17β-HSD2 regulates the levels of testosterone and estradiol that activate the androgen receptor and estrogen receptor, respectively. Interestingly, although human 11β-HSD2 and 17β-HSD2 act at different positions on different steroids, these enzymes are paralogs. Despite the physiological importance of 11β-HSD2 and 17β-HSD2, details of their origins and divergence from a common ancestor are not known. An opportunity to understand their evolution is presented by the recent sequencing of genomes from sea urchin, a basal deuterostome, and amphioxus, a basal chordate, and the availability of substantial sequence for acorn worm and elephant shark, which together provide a more complete dataset for analysis of the origins of 11β-HSD2 and 17β-HSD2. BLAST searches find an ancestral sequence of 17β-HSD2 in sea urchin, acorn worm and amphioxus, while an ancestral sequence of 11β-HSD2 first appears in sharks. Sequence analyses indicate that 17β-HSD2 in sea urchin may have a non-enzymatic activity. Evolutionary analyses indicate that if acorn worm 17β-HSD2 is catalytically active, then it metabolizes novel substrate(s).

Introduction. 11β-hydroxysteroid dehydrogenase type-2 (11β-HSD2) and 17β-HSD2 catalyze the conversion of C11 and C17 alcohols, respectively, to ketones on glucocorticoids, androgens and estrogens [Figure 1], which makes these enzymes important partners with steroid receptors in the regulation of steroid hormone action [1,2,3,4]. Tissue-specific expression of 11β-HSD2 and

17β-HSD2 allows these enzymes to act as gatekeepers in regulating access of active glucocorticoids, androgens and estrogens to their receptors. For example, by metabolizing cortisol to cortisone, an inactive steroid, 11β-HSD2 regulates the concentration of cortisol that can activate the glucocorticoid receptor [GR] and mineralocorticoid receptor [MR] [4,5,6,7]. Interestingly, in fish, 11β-HSD2 catalyzes the conversion of 11β-hydroxy-testosterone to 11-keto-testosterone [11-keto-T], which is the active androgen in fish [8,9,10].

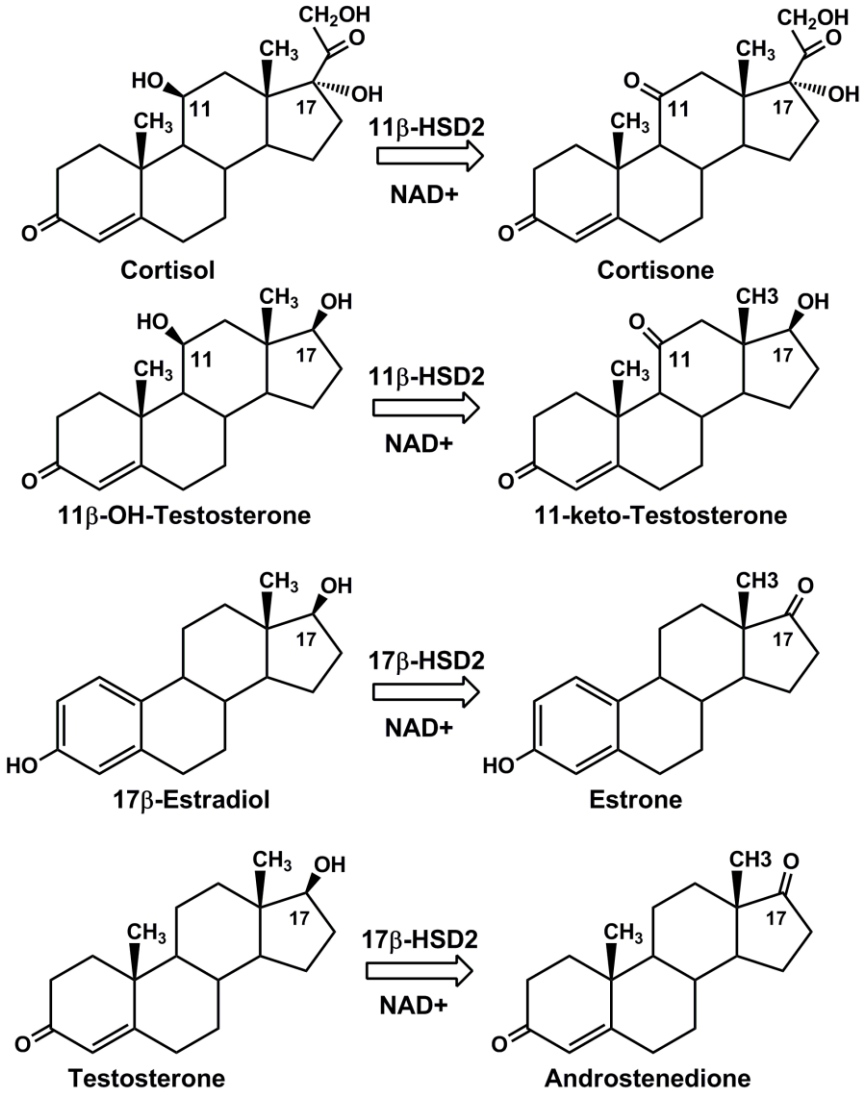

Figure 1. Reactions catalyzed by 11β-HSD2 and 17β-HSD2. 11β-HSD2 and 17β-HSD2 are NAD+-dependent enzymes that metabolize the C11-alcohol and C17-alcohol, respectively, on steroids.

Similarly, tissue-specific expression of 17β-HSD2 regulates access of active androgens and estrogens to the androgen receptor [AR] and estrogen receptor [ER] [1,2,3]. Thus, along with

the AR and ER, 17β-HSD2 has a key role in the physiological actions of androgens and estrogens.

Sequence analyses of 17β-HSD2 and 17β-HSD2 reveal that they belong to the short chain dehydrogenases/reductases [SDR], which is a large and diverse family of enzymes that are found in bacteria, plants and animals [11,12,13,14]. Previous evolutionary analyses reveal that 11β-HSD2 and 17β-HSD2 are found in fish and land vertebrates [2,3,15,16,17,18,19,20]. However, several questions remain regarding more ancient events in the evolution of 11β-HSD2 and 17β-HSD2. When did these enzymes evolve; which enzyme evolved first, and when did the second enzyme first appear in animals? Also, what was the substrate(s) of the ancestral enzyme? That is, did the common ancestor of 11β-HSD2 and 17β-HSD2 metabolize glucocorticoids or androgens or estrogens or a combination of these steroids or did it metabolize a different hormone?

This is an opportune time to seek answers to these questions about the origins and evolution of 11β-HSD2 and 17β-HSD2 because the genomes from sea urchin (*Strongylocentrotus purpuratus*), a basal deuterostome and amphioxus (*Branchiostoma floridae*), a basal chordate, have been sequenced. In addition, recently, many sequences from the acorn worm (*Saccoglossus kowaleski*), another basal deuterostome, were deposited in GenBank, and a draft of the elephant shark genome [21] and lamprey genome also are available [Figure 2].

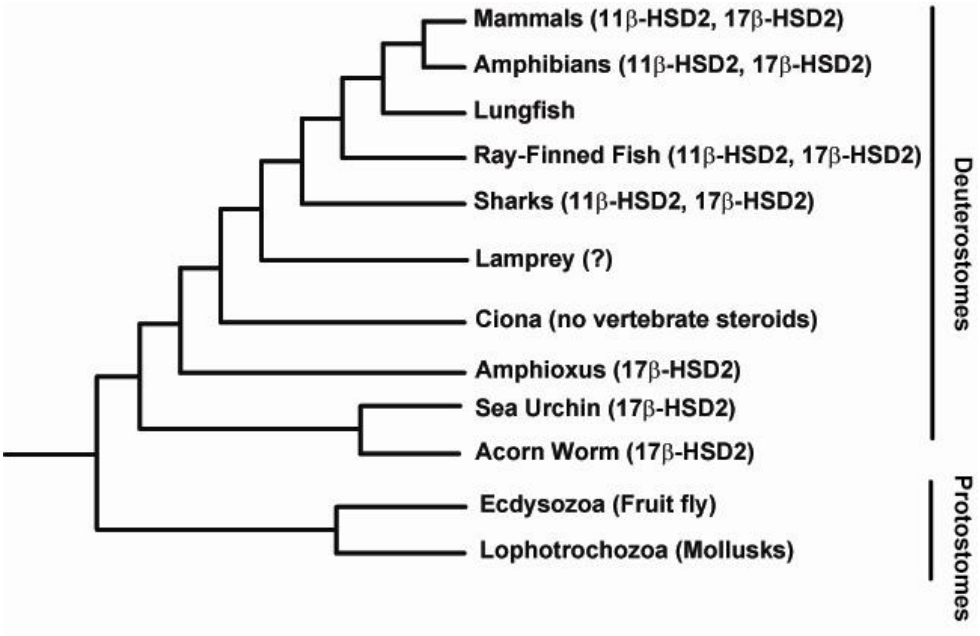

Figure 2. Phylogeny of deuterostomes and protostomes [22]. Sea urchin and acorn worm contain a 17β-HSD2 ortholog. Steroid receptors are not found in sea urchin [29,54]. 11β-HSD2 is in elephant shark, but not in amphioxus or Ciona. It is not known if either 11β-HSD2 or 17β-HSD2 is present in lamprey.

As a result, a more complete genomic dataset can be used for analysis of the origins of 11β-HSD2 and 17β-HSD2. With this in mind, we searched GenBank and other databases with 11β-HSD2 and 17β-HSD2 for orthologs. These searches found an ortholog of 17β-HSD2 in sea urchin, acorn worm and amphioxus, while 11β-HSD2 first appears in the elephant shark. This indicates that 17β-HSD2 is the ancestor of 11β-HSD2 and suggests that 17β-HSD2 and 11β-HSD2, respectively, may have had a role in evolution of basal deuterostomes [22] and sharks.

Methods

We used the sequences for human 11β-HSD2 [Accession: **P80365**) and human 17β-HSD2 [Accession: **P37059**) to search GenBank (www.ncbi.nlm.nih.gov/entrez), Ensembl (http://www.ensembl.org/), elephant shark server (http://esharkgenome.imcb.a-star.edu.sg/), Sea Urchin Genome Database (http://www.spbase.org/SpBase), Joint Genome Initiative Database (http://genome.jgi-psf.org/) and Gene Indices (http://compbio.dfci.harvard.edu/tgi/tgipage.html) with BLAST [23] for orthologs. The accessions for each gene are presented in Table 1 [supplement].

Multiple alignments of 11β-HSD2 and 17β-HSD2 were done with Clustal X using the iteration option for each step in the multiple alignment [24,25]. This alignment was converted to a phylogenetic tree using the neighbor-joining algorithm with a correction of branch lengths for rate heterogeneity between sites [26].

Results

Identification of orthologs of 11β-HSD2 and 17β-HSD2

Analysis of the evolution of 11β-HSD2 and 17β-HSD2 depends on tracing their orthologs in multi-cellular animals. Two genes are orthologs, if they have evolved only by speciation events [27]. Thus, human and mouse 11β-HSD2 are orthologs. In contrast, human 11β-HSD2 and 17β-HSD2 are paralogs, having evolved through a gene duplication and divergence to yield enzymes with two different catalytic activities. The amino acid sequences of human 11β-HSD2 and 11β-HSD2 are about 41% identical over 341 amino acids, with 14% conservative replacements and only one gap. This difference in their sequences is not surprising because glucocorticoids and estrogens have different functional groups at C11 and C17 [Figure 1]. Nevertheless BLAST searches show that 11β-HSD2 and 17β-HSD2 are closest to each other in

GenBank. That is, a search with 11β-HSD2 finds a series of 11β-HSD2 orthologs in vertebrates, followed by a series of vertebrate 17β-HSD2 paralogs. A similar BLAST search with 17β-HSD2 finds a series of 17β-HSD2 orthologs, followed by 11β-HSD2 paralogs.

Phylogenetic analysis of 11β-HSD2 and 17β-HSD2.

Proteins identified with BLAST as being possible orthologs of 11β-HSD2 or 17β-HSD2 were collected for the phylogenetic analysis, shown in Figure 3. This analysis reveals that 17β-HSD2 has orthologs in sea urchin, acorn worm and amphioxus. Both 11β-HSD2 and 17β-HSD2 are found in fish and land vertebrates, as has been previously reported [2,3,15,16,17,19,20]. BLAST searches of the elephant shark genome found partial sequences of 11β-HSD2 and 17β-HSD2 [Supplement Table 2], but the sequences were not long enough to be included in the phylogenetic analysis. BLAST searches did not find either 11β-HSD2 or 17β-HSD2 in Ciona, which lacks steroid receptors [28,29], or in sea lamprey. Although there is substantial coverage of the sea lamprey genome, it is incomplete. Thus, the absence of 11β-HSD2 and 17β-HSD2 from lamprey is not yet established. With this caveat, it appears that 11β-HSD2 arose in a shark, after 17β-HSD2 arose in a basal deuterostome [22].

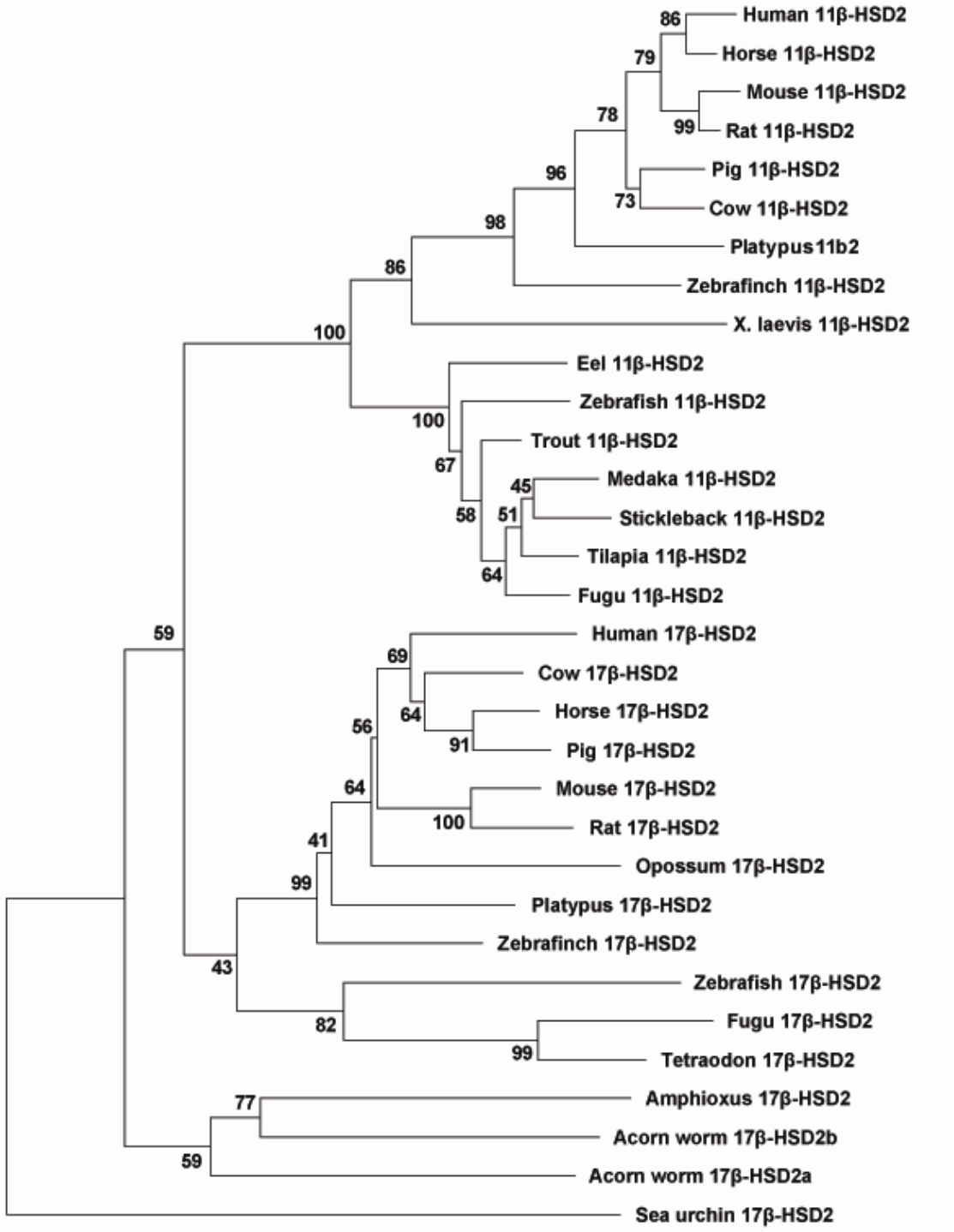

Figure 3. Phylogenetic analysis of 11β-HSD2 and 17β-HSD2. Clustal X [24,25] with 1,000 bootstrap trials was used to construct the phylogenetic tree [26]. Branch lengths are proportional to the distances between each protein. Bootstrap values are the percent of trials that this cluster was found in 1,000 trials. BLAST searches did not find 11β-HSD2 in opossum, which also appears to lack 11β-HSD1 [59]. Tetraodon contains a partial sequence of 11β-HSD2. BLAST searches did not find 17β-HSD2 in *Xenopus*.

Unusual amino acids in the catalytic site of amphioxus and sea urchin 17β-HSD2.

11β-HSD2 and 17β-HSD2, like other SDRs, contain a triad consisting of tyrosine, lysine and serine at the catalytic site [11,13]. A highly conserved asparagine also is important in catalysis [30]. In Figure 4 we show a multiple alignment of the amphioxus, acorn worm and sea urchin 17β-HSD2 orthologs with human 17β-HSD2. Inspection of this alignment reveals that all of these 17β-HSD2s have conserved the asparagine and lysine residues. The two acorn worm 17β-HSD2s have the key serine and tyrosine residues. However, amphioxus 17β-HSD2 has a glutamic acid instead of a tyrosine, and sea urchin 17β-HSD2 has a phenylalanine and glycine, respectively, instead of a tyrosine and serine. This suggests unusual properties of the amphioxus and sea urchin 17β-HSD2s.

Inspection of Figure 4 reveals that there are forty positions in which all five amino acids are identical, and forty two positions in which four out of five amino acids are identical. This is remarkable sequence conservation, considering that 17β-HSD2 evolved over 500 million years ago [22]. These conserved sites are likely to have important functional or structural roles in human 17β-HSD2 and 11β-HSD2.

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Human 11β-HSD2          1 -----------MERWPWPSGGAWLLVAARALLQ--LLRSDLRLGRPLLAALALLA
Human 17β-HSD2          1 -----------MS--TFFSDTAWICLAVPTVLCGTVFCKYKKSSGQLWSWMVCLA
AcornW 17β-HSD2b        1 MEESLSEGGDEELIMLYHLVYSTVSFIFGIAVLVKYFRNELRLGVRCVYALLVLF
Amphioxus 17β-HSD2      1 --------------MIYQLLYSVVALCFGVSVFVRYVNDGMNFGFRSALSLLVLF
AcornW 17β-HSD2a        1 ------------MANVGQIIVRYILFIL-LGIFVYIIYDSLFPLVPIGKSKFAFS
Sea urchin 17β-HSD2     1 -------------------------------KVQVGSLDGKVGSSLRSMRVHTRVN

Human 11β-HSD2         43 ALDWLCQRLLPPPAALAVLAAAGWIALSRLARPQRLPVATRAVLITGCDSGFGKE
Human 17β-HSD2          43 GLCAVCLLILSPFWGLILFSVSCFLMYTYLSGQELLPVDQKAVLVTGGDCGLGHA
AcornW 17β-HSD2b        56 AGEPVCHFAIGSHLGVIIFILFCIVTYVTLP-ANHLEINHKAVLVTGCDSGLGLA
Amphioxus 17β-HSD2      42 LGEPFCHHVVGEVEGLLLYGLACVVMYLLLP-AGEVVLKDRVVLITGCDSGFGRA
AcornW 17β-HSD2a        43 LTPVPFKASIG---VVIIIG----VWIYMLP-AKRLEVGRKSVLVTGCDSGFGHA
Sea urchin 17β-HSD2     26 HGAKQCLHFETRLTAYCIQS----VLYQLLS---------QPETDRGCESGLAHS
                                                  *           :: :*:::* :

Human 11β-HSD2          98 TAKKLDSMGFTVLATVLELNSPGAIELRTCCSPRLRLLQMDLTKPGDISRVLEFT
Human 17β-HSD2          98 LCKYLDELGFTVFAGVLNENGPGAEELRRTCSPRLSVLQMDITKPVQIKDAYSKV
AcornW 17β-HSD2b       110 LAQYLDKRGFDVFAGILHKGGHGEVLLNASCSTRLTTLQLDVTKKDQIQQAFQTV
Amphioxus 17β-HSD2      96 LAQHLDSLGCVVFAGCLQ--GEGATSLKSSCSDQLKILQLDVTDAQQVEQARQVV
AcornW 17β-HSD2a        90 LAIYLDLLGFHVFAGCLFKDGEGAKELRNMCSERLTILQFDVTSQIQVDNALEEV
Sea urchin 17β-HSD2     68 TAAHLDSLGFTVYAAFLTEDAKAEKELAKLCSDRLVTLYLDPSDDNRVTSTVEEL
                            :  **  *: * *  *   : :   *   **  *  *: * :

Human 11β-HSD2         153 KAHTTS-TGLWGLVNNAGHNEVVADAELSPVATFRSCMEVNFFGALELTKGLLPL
Human 17β-HSD2          153 AAMLQD-RGLWAVINNAGVLGFPTDGELLLMTDYKQCMAVNFFGTVEVTKTFLPL
AcornW 17β-HSD2b        165 QRKLGG-QGLWGLVNNAGVFSFM-DIELTPLSIYQQLLDVNCLAVVQMTKTFLPL
Amphioxus 17β-HSD2      149 QEYLGPDRGLWGLVNNAGLVYFG-ELDLLPFSMVQHSIDVNLVGMLRMTKTFLPV
AcornW 17β-HSD2a        145 NNKLK--GELWGLVNNAGILHTS-EFELTPTSVMEQTMEINCLGPMRVTKAFLPL
Sea urchin 17β-HSD2     123 RESLNG-NDLWGLVNGVTCCYYA-ESEIMPLDFYRKLWSVNVEGQIRVTRALLPL
                               :     **:::*:::        :: :         :*  :    *:  **:

Human 11β-HSD2          311 LRSSRGRIVTVGSPAGDMPYPCLGAYGTSKAAVALLMDTFSCELLPWGVKVSIIQ
Human 17β-HSD2          207 LRKSKGRLVNVSSMGGGAPMERLASYGSSKAAVTMFSSVMRLELSKWGIKVASIQ
AcornW 17β-HSD2b        218 IRKVQGRVVNISSTYGEVPLMGLSAYNISKAGVEIFTDVLRQEMKKWGIKVSLVE
Amphioxus 17β-HSD2      203 LRKGKGRVINVSSIAGSVPLMFMCAEGAAKAGVEAASHILRQELKKWGVHVSIVQ
AcornW 17β-HSD2a        197 IRNSKGRIVNVASIAGRVCVGLHTVYAASKAGVELFSDGLRQEMVKWGVNVSIIE
Sea urchin 17β-HSD2     176 LRRSRGRIVNITGISNRLPAPGFSAFCASKAALLMYSDVLRLEMRKWGVHVSVIE
                             *   ** ::     :  :         :** :       : *  :**  *:

Human 11β-HSD2          311 PGCFKTESVRNVGQWEKRKQLLLANLPQELLQAYGKDYIEHLHGQFLHS------
Human 17β-HSD2          262 PGGFLTNIAGTSDKWEKLEKDILDHLPAEVQEDYGQDYILAQRNFLLLI------
AcornW 17β-HSD2b        273 PYGFLTSII-SRDKMDCVYHSMMDNLTPELLQLYGTSYFETYKKEIVEHEGRTLH
Amphioxus 17β-HSD2      258 PFAFKTEWQ-SSESMLKNYNQLRQALDKDTLDTYGLDYLDAFKHNLLNE------
AcornW 17β-HSD2a        252 PAGFKTTLF-SEKALESMYKSVKENMTPTVKKDYGEAYMKSFQDSLMSLSKMP--
Sea urchin 17β-HSD2     231 PSEYRAAFN-KDGRLKKALHHMTHELSPLVSRDYGDQYFDAAMATVDRED-----
                             *  : :                   :       **  *

Human 11β-HSD2          311 -LRLAMSDLTPVVDAITDALLAARPRRRYYPGQGLGLMYFIHYYLPEGLRRRFLQ
Human 17β-HSD2          311 -NSLASKDFSPVLRDIQHAILAKSPFAYYTPGKGAYLWICLAHYLPIGIYDYFAK
AcornW 17β-HSD2b        327 CSESLTEDCQFLCRCVRDALLSRWPQARYPEGPGARIIMLLSDHLPTAMLDLFLP
Amphioxus 17β-HSD2      306 --ESLNEDLGPVIGVMTDALTSRGPRAWYPCGRGTRSLVWLSNLVPTSILDMILP
AcornW 17β-HSD2a        304 -KKYLPEDISPVIDSIYDALLSTQPKPRYTIGTGKWFMLFIGNCLPTKIGDRFLL
Sea urchin 17β-HSD2     280 -GTDFTPDQSAPCKAIEHALLAKNPDEHYSCDKTSIVLGALTSHAPAFITDTIAK
                                    *          *::   *   *  :             *

Human 11β-HSD2          365 AFFIS-HCLPRALQPGQPGTTPPQDAAQDPNLSPGPSPAVAR-------------
Human 17β-HSD2          365 RHFGQDKPMPRALR--------------MPNYKKKAT-----------------
AcornW 17β-HSD2b        382 HILPDWAKIKPDVSQSSSNMQY-----------------------------------
Amphioxus 17β-HSD2      359 LLMLQIDVTPTLLRER-------------------------------------
AcornW 17β-HSD2a        358 KLAPKTLLPAALFNQNKNKDV---------------------------------
Sea urchin 17β-HSD2     334 FLAGTQISERCLAMTSEDKDSGNSRKAADDYKNDNVESSKDVTNNINAHNYPVTT
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Figure 4. Alignment of 11β-HSD2 and 17β-HSD2. Clustal X was used to align human, amphioxus, sea urchin and acorn worm 17β-HSD2 and human 11β-HSD2. Tyrosine, lysine serine and asparagine that are functionally important in SDRs[11,13,30] are shown in green. Sea urchin 17β-HSD2 contains a phenylalanine, which is a conservative replacement of tyrosine. However, phenylalanine lacks the hydroxyl in tyrosine, which suggests that sea urchin 17β-HSD2 is not catalytically active. Amphioxus 17β-HSD2 contains a glutamic acid and glycine, respectively, instead of the functional tyrosine and serine.

Discussion

The recent sequencing of the genomes for amphioxus and sea urchin and the availability of a substantial set of sequences from the acorn worm, lamprey and elephant shark has provided data that permits a deeper analysis of the origins and diversification of 11β-HSD2 and 17β-HSD2. The phylogenetic analysis of 11β-HSD2 and 17β-HSD2 presented here [Figure 3] provides insights into the evolution of the enzyme-mediated mechanism for regulating steroid hormone activation of steroid receptors, as well as raising questions for future research about the functions of 17β-HSD2 in sea urchin, acorn worm and amphioxus, and the relationship of 17β-HSD2 to retinol dehydrogenases that metabolize estrogens and androgens [16,17,20,31,32].

Evolution of 11β-HSD2.

The evidence that 11β-HSD2 evolved in a shark, or possibly earlier in lamprey, has implications for the origins of the enzyme-mediated mechanism for selective temporal and spatial regulation of glucocorticoid access to the GR and MR in vertebrates [4,5,6,7] and 11-keto-T to the AR in fish [8,9,10]. Evolution of 11β-HSD2 in shark and not in amphioxus is consistent with the presence in sharks of a steroids such as corticosterone and 1α-hydroxy-corticosterone, which have a functionally important C11-hydroxyl [33,34,35,36] and the absence of glucocorticoids in amphioxus [37].

The absence of an ortholog for 11β-HSD2 in lamprey is perplexing because much of the lamprey genome has been sequenced and a search with 11β-HSD2 would be expected to retrieve at least a partial sequence. However, because the current lamprey database is incomplete, it is possible that a lamprey 11β-HSD2 will be found after the sequencing of the lamprey genome is completed. Another way to address this question is to determine if lamprey contains

11β-hydroxy-steroids, such as cortisol and corticosterone, which would be substrates for a lamprey 11β-HSD2. In this regard, there is conflicting evidence for the presence of cortisol and corticosterone in lamprey [reviewed in [38]]. There are reports that cortisol and corticosterone are not synthesized by lamprey [39,40]. Other reports provide evidence for cortisol and corticosterone in lamprey [41,42]. Further studies with lamprey are needed to resolve this enigma.

Evolution of 11β-HSD2 as a regulator of the mineralocorticoid response

A key element for11β-HSD2 acting as a gatekeeper to prevent cortisol or corticosterone activation of the MR is that aldosterone, the physiologically important mineralocorticoid in land vertebrates, is not metabolized by 11β-HSD2 [4,5,6,7]. Neither shark nor bony fishes contain aldosterone, which first appears in lungfish [43]. In fish, DOC and cortisol are potent mineralocorticoids [33,44,45,46]. Unlike cortisol, DOC lacks a C11-hydroxyl, and, thus, like aldosterone, is inert to 11β-HSD2 [33,47,48]. If 11β-HSD2 first appeared in sharks, then this marks the evolution of the mechanism for local expression of 11β-HSD2 to prevent access of glucocorticoids, such as corticosterone and 1α-hydroxy-corticosterone, to the MR, which still would respond to DOC or 11-deoxycortisol [34,35,36]. The appearance in shark of this gatekeeper mechanism for regulating glucocorticoid and mineralocorticoid access to the GR and MR may have been important in the transition from jawless fish to sharks.

Evolution of 17β-HSD2.

In sharks, fish and land vertebrates, 17β-HSD2 should regulate access of estrogens and androgens to the ER and AR [1,2,3]. However, the functions of 17β-HSD2 in amphioxus, sea urchin and acorn worm are not as clear because, of these three animals, only amphioxus has been shown to have steroid receptors [49,50,51,52] and sex steroids [37,53]. Amphioxus contains receptors with sequence similarity to the ER and the 3-keto-steroid receptor [SR]. The presence of an ortholog of 17β-HSD2 in amphioxus is consistent with the evidence for the synthesis of androgens and estrogens in amphioxus [37,53] and transcriptional activity of estrogens in amphioxus [49,52]. However, amphioxus 17β-HSD2 has a glutamic acid instead of a tyrosine that is found in the catalytic site of most SDRs [11,13]. The pKa of the γ-carboxyl on glutamic acid is about 4.3, in contrast to the pKa of about 10 for the phenolic hydroxyl on tyrosine. This suggests that amphioxus 17β-HSD2 may be catalytically active at neutral pH or below, in response to unique physiological conditions for androgen and estrogen action in amphioxus.

Sea urchin does not contain an ortholog of the AR or ER [54], which suggests that sea urchin 17β-HSD2 metabolizes other substrates. A complication is that sea urchin 17β-HSD2 may not be catalytically active because it contains a phenylalanine, which lacks a C3-hydroxyl and, thus, would not be able to substitute for tyrosine in metabolizing alcohols on substrates. Meier et al. [55] found that hydroxysteroid dehydrogenase like 1 protein [HSDL1], which is an SDR, has a phenylalanine instead of a tyrosine in the catalytic site. They showed that human HSDL1 interacts with a map kinase phosphatase-like protein. It may be that sea urchin 17β-HSD2 also has a non-catalytic activity that involves binding to another protein. This possibility for sea urchin 17β-HSD2 needs to be investigated.

Acorn worm contains two 17β-HSD2 homologs that have the conserved tyrosine, lysine, serine and asparagine, and, thus, both enzymes could be catalytically active. However, if, acorn worm, like the sea urchin, does not contain either an ER or an AR [29] or steroidogenic enzymes [29], then acorn worm 17β-HSD2s would be expected to have other activities. Previous phylogenetic analyses reveal that 17β-HSD2 is close to retinol dehydrogenases [2,16,18,20,56], some of which metabolize retinol, estradiol and testosterone [16,31,32,57,58]. Acorn worm 17β-HSD2 may metabolize retinol or perhaps a C3-alcohol on sterol or bile acid.

A perplexing finding is that BLAST searches find orthologs of 17β-HSD2 only in zebrafish, Fugu and Tetraodon, in contrast to 11β-HSD2, which is found in several fish, including zebrafish, tilapia, trout, eel, medaka, Fugu and Tetraodon [Figure 3], [18]. The paucity of fish 17β-HSD2 was first reported by Mindnich et al [18] as part of a study in which they showed that zebrafish 17β-HSD2 is inactive. They searched databases from December 2005 for othologs of 17β-HSD2. It is significant and perplexing that, despite the substantial expansion of GenBank since 2005, BLAST does not retrieve other fish 17β-HSD2s from GenBank.

The evidence that zebrafish 17β-HSD2 lacks catalytic activity [18] is clear and, also perplexing. It is not known if 17β-HSD2 from Fugu or Tetraodon has catalytic activity. At this time, the protein responsible for 17β-HSD2 activity in fish remains unknown. Perhaps, one of the retinol dehydrogenases performs this function [16,31,32,57,58].

Thus, although the availability of substantial sequence information for sea urchin amphioxus, lamprey and shark provide data that clarifies the origins of 11β-HSD2 and 17β-HSD2, there remain important questions that need to be answered regarding the functions of these

enzymes in fish, lamprey, amphioxus and their ancestors in basal deuterostomes [22].

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Table 1. Accessions for 11β-HSD2 and 17β-HSD2

Gene	Accession
Human 11β-HSD2	NP_000187
Horse11β-HSD2	NP_001075395
Mouse 11β-HSD2	NP_032315
Rat 11β-HSD2	NP_058777
Pig 11β-HSD2	NP_999078
Cow 11β-HSD2	AAI02489
Zebrafinch 11β-HSD2	XP_002187491
Opossum 11β-HSD2	Not found in GenBank
Platypus 11β-HSD2	XP_001512268
Xenopus laevis 11β-HSD2	NP_001086062
Eel 11β-HSD2	116267595
Zebrafish11β-HSD2	NP_997885
Trout 11β-HSD2	NP_001117690
Medaka 11β-HSD2	ABK59971
Stickleback11β-HSD2	ENSGACT00000023181*
Tilapia 11β-HSD2	AAO42610
Fugu 11β-HSD2	ENSTRUP00000019011*
Tetraodon 11β-HSD2	47214187
Elephant Shark 11β-HSD2	AAVX01118039, AAVX01131585
Human 17β-HSD2	NP_002144
Cow 17β-HSD2	NP_001069194
Horse17β-HSD2	XP_001916492
Pig 17β-HSD2	NP_001161121
Mouse 17β-HSD2	NP_032316
Rat 17β-HSD2	NP_077367
Opossum 17β-HSD2	XP_001380985
Platypus 17β-HSD2	XP_001508240
Zebrafinch 17β-HSD2	XP_002194634
Xenopus laevis 17β-HSD2	Not found in GenBank
Zebrafish17β-HSD2	NP_001035278
Fugu 17β-HSD2	ENSTRUP00000019011*
Tetraodon 17β-HSD2	ENSTNIT00000023208*
Elephant Shark 17β-HSD2	AAVX01196666, AAVX0148010
Amphioxus 17β-HSD2	XP_002608268
Acorn worm 17β-HSD2a	XP_002741661
Acorn worm 17β-HSD2b	XP_002735924
Sea Urchin 17β-HSD2	SPU_021966**

Accessions from *Ensembl: www.ensembl.org, **sea urchin server: www.spbase.org/SpBase, and ***elephant shark server: esharkgenome.imcb.a-star.edu.sg. All other accessions from GenBank: www.ncbi.nlm.nih.gov/entrez.

Supplement Table 2. Partial sequences of Elephant Shark 11β-HSD2 and 17β-HSD2

A. Partial sequence of elephant shark 11β-HSD2
>Accession AAVX01118039
SLTFAGCDSGFGKTIAQHFDSMGFKVFATVLNKDGPGAIELVQMCSEELTLIQMDLT
KPQ
DIENAVQFTK

>Accession AAVX01131585
VSFSTGEIPFGRMSAYGSSEAALELYSDILRQEMKIWGVKVSIIQPGATKT

>elephant shark 11β-HSD2
SLTFAGCDSGFGKTIAQHFDSMGFKVFATVLNKDGPGAIELVQMCSEELTLIQMDLT
KPQ
DIENAVQFTK
VSFSTGEIPFGRMSAYGSSEAALELYSDILRQEMKIWGVKVSIIQPGATKT

B. Partial sequence of elephant shark 17β-HSD2
>Accession AAVX01196666
LYCSACPLRSANSDLLPISGRA

>Accession AAVX0148010
GNLPLMGFAAYGASKAALSRFSEVLRQEPSQWGIKVATIQTSGFKTGL

>elephant shark 17β-HSD2
LYCSACPLRSANSDLLPISGRA
GNLPLMGFAAYGASKAALSRFSEVLRQEPSQWGIKVATIQTSGFKTGL