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Evolution of adrenal and sex steroid action in vertebrates: a ligand-based mechanism for complexity

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Summary

Various explanations have been proposed to account for complex differentiation and development in humans, despite the human genome containing only two to three times the number of genes in invertebrates. Ignored are the actions of adrenal and sex steroids—androgens, estrogens, glucocorticoids, mineralocorticoids, and progestins—which act through receptors that arose from an ancestral nuclear receptor in a protochordate. This ligand-based mechanism is unique to vertebrates and was integrated into the already robust network of transcription factors in invertebrates. Adrenal and sex steroids influence almost all aspects of vertebrate differentiation and development. I propose that evolution of this ligand-based mechanism in a primitive vertebrate was an important contribution to vertebrate complexity. Sequencing of genomes from a cephalochordate, such as amphioxus, and from hagfish and lamprey will establish early events in the evolution of steroid hormone signaling, and also allow genetic studies to elucidate how vertebrate complexity depends on steroid hormones.

Introduction

One of the most intriguing and humbling discoveries to arise from sequencing of the human genome is that it consists of about 35,000 genes [1,2], with only about 7,000 genes that appear to be unique to the vertebrate line. This is much less than previous estimates of 80,000 to 140,000 genes and not much more than ~18,000 genes in *Caenorhabditus elegans* and ~13,000 in *Drosophila melanogaster*. Various rationales have been proposed to explain the unexpected low gene number in humans. First, gene number is not as low as it seems because

about 40% of human genes are alternatively spliced. Second, human proteins contain novel combinations of domains, which allow for increased protein–protein interactions, compared to invertebrate proteins [1-3]. Third, posttranslational modification of proteins adds complexity. Fourth, the evolution in vertebrates of networks of transcription factors and the genes that they regulate [4].

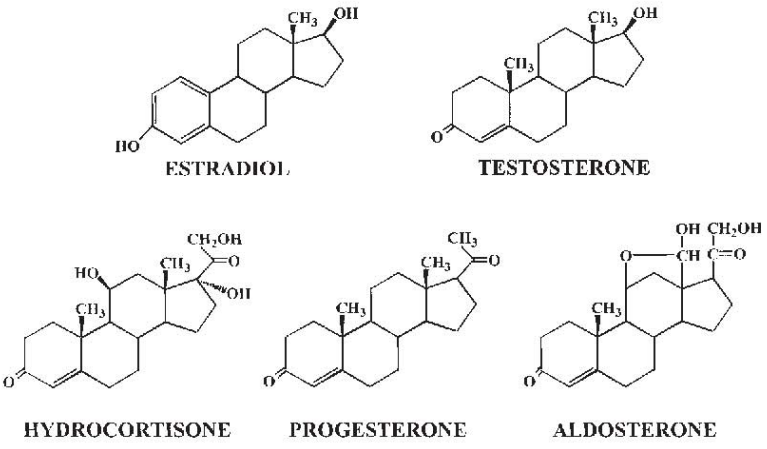

Figure 1. Structures of adrenal and sex steroids.

To these I add the evolution in chordates of receptors for adrenal and sex steroids: aldosterone, estradiol, hydrocortisone, progesterone and testosterone (Fig. 1) [5-8], which is a ligand-based based mechanism for regulating the interactions between networks of transcription factors [9-11], as well as directly regulating the transcription of specific genes. Receptors for these steroids have not been found in invertebrates. In vertebrates, these steroids regulate a wide range of physiological processes including reproduction, differentiation, development and homeostasis. I propose that the emergence in vertebrates of this ligand-based mechanism for regulating gene transcription, combined with regulation of steroid hormone access to their receptors by enzymes (Fig. 2) [12-17], was an important element in complex differentiation and development found in humans and other vertebrates.

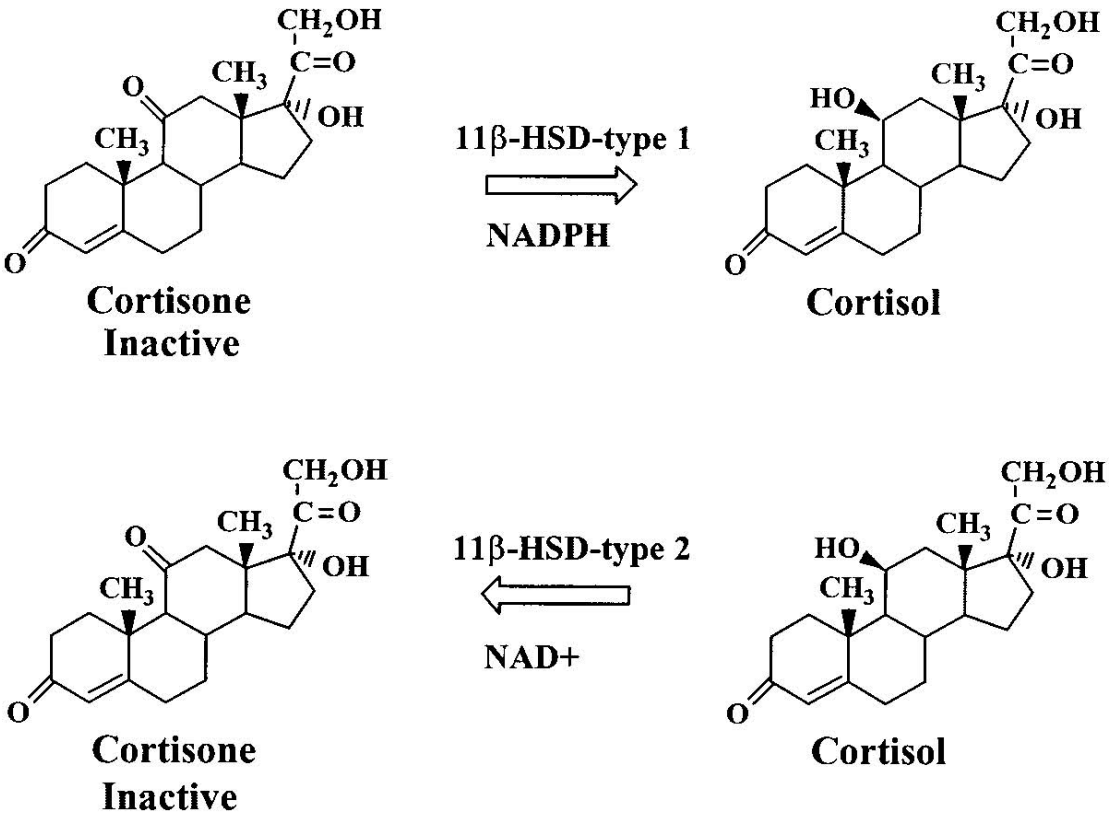

Figure 2. Regulation of mineralocorticoid action in the kidney by 11β-hydroxysteroid dehydrogenase-type 2. In mammals, 11β-hydroxysteroid dehydrogenase-type 1 (11β-HSD-type 1) and 11βHSD-type 2, which have less than 20% sequence identity, preferentially catalyze the reduction and oxidation of a ketone and alcohol, respectively, at C11 on glucocorticoids. Both aldosterone and cortisol can regulate sodium and potassium transport in the distal tubule of the kidney. Selective expression of 11β-HSD-type 2 in the distal tubule of the kidney results in conversion of cortisol to cortisone, an inactive steroid. However, 11βHSD-type 2 does not metabolize aldosterone, which can bind to the mineralocorticoid receptor and regulate electrolyte transport.

Steroid receptors arose in a protochordate and/or primitive vertebrate

Although nuclear receptors are found in vertebrates and invertebrates, to date, only vertebrates have been found to express adrenal and sex steroid receptors. An ER, PR and corticoid receptor (CR) have been cloned from lamprey, a jawless fish [18,19]. It appears that the ER is the most ancient of the adrenal and sex steroid receptors [8,18].

The DNA-binding and ligand-binding domains of lamprey ER are 89% and 64% identical to human ER-alpha (not shown), indicating strong sequence conservation during the ~525 million years since humans and jawless fish separated from a common ancestor. This

sequence conservation suggests that it should be possible to identify an ancestral estrogen receptor in a genome of a protochordate such as *Ciona intestinalis*. A BLAST search of the *C. intestinalis* genome (http://www.tigr.org/tdb/tgi/cingi) with the DNA-binding and ligand-binding domains of human ER and a proposed ancestral ER [18] did not find any *Ciona* genes with convincing sequence similarity; for example, at least 80% sequence identity in the DNA-binding domain. Similar BLAST searches of the *Ciona* genome with human PR and human GR and an ancestral 3-keto-steroid receptor [18] did not identify a steroid receptor ortholog. These data lead me to propose that adrenal and sex steroid receptors arose in a cephalochordate, such as amphioxus, after separation of urochordates from the chordate line (Fig. 3).

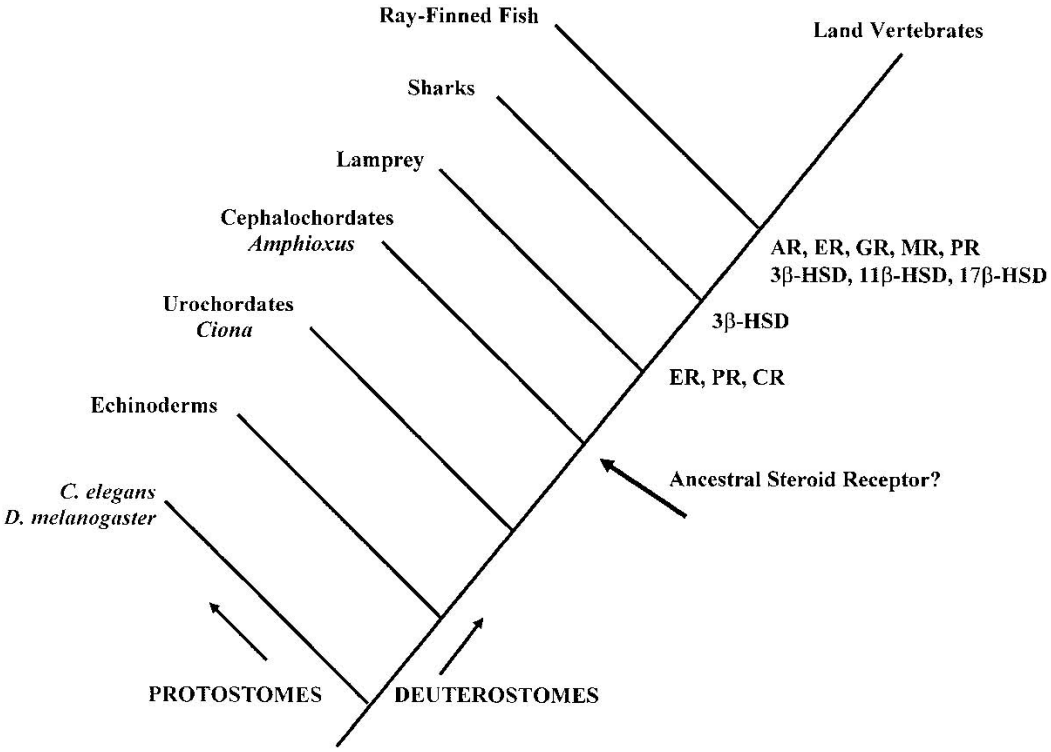

Figure 3. Evidence for steroid receptors and hydroxysteroid dehydrogenases in chordates. Genes for steroid receptors and enzymes that regulate steroid hormone concentrations have been found in fish and land vertebrates. Lamprey, a jawless fish, contains orthologs of ER, PR and GR [18]. A BLAST search of *F. rubripes* genome found putative ancestors of 11β-HSD-types 1 and 2 and 17β-HSD-types 1,2 and 7. Nunez and Trant found an ortholog of $3\beta/\Delta^{5-4}$-HSD in southern stingray, a cartilaginous fish. Our recent BLAST searches indicate that steroid receptors and 11β-HSD-types 1 and 2, 17β-HSD-types 1,2 and $3\beta/\Delta^{5-4}$-HSD are not present the Ciona genome.

An alternative possibility is that *Ciona* has lost gene(s) for steroid receptor(s) that evolved in an ancestral urochordate or in an echinoderm. Although gene loss is common in chordates [20,21], usually such events occur when there are multiple copies of the gene; in which case, loss of one or more redundant genes for a transcription factor does not abolish its actions. In contrast, loss of all receptors that mediate steroid action would be expected to have severe consequences in the host, assuming that the steroid response is important in the organism. The sequencing of genomes from other urochordates, echinoderms and cephalochordates will help to clarify where in the chordate line steroid receptors first evolved and if these genes have been lost in *Ciona*.

Steroids regulate gene transcription in many tissues in vertebrates

Subsequent duplications of this receptor gave rise to a family of steroid receptors, possibly during genome size or chromosomal duplications that are thought to have occurred before and after the evolution of jawless fish [20,21]. By that time, robust regulation of differentiation, development and homeostasis had evolved in multicellular animals [22]. The steroid response was integrated into these pathways, with steroids assuming key roles in developmental complexity. Examples are the regulation by androgens and estrogens of sexual differentiation and development in vertebrates and glucocorticoid actions on the immune and nervous systems.

Moreover, adrenal and sex steroids act in many tissues that are not usually thought of as classical target tissues. Thus, estrogen, in addition to acting in the ovary to regulate egg maturation, regulates gene transcription in bladder, bone, brain, heart, kidney, liver, lung, pituitary, prostate, spinal chord, spleen, testis and thymus [23]. Similarly, androgens, cortisol and progesterone regulate gene transcription in the brain, heart, kidney and liver as well as other tissues.

The steroid response also cross-talks with responses mediated by proteins that arose in invertebrates [22,24]. Examples are insulin-like growth factor, epidermal growth factor, and transforming growth factor-beta, which act by binding to receptors on the cell membrane, and c-Fos and c-Jun, transcription factors that bind to AP-1 sites [9-11,25]. Cross-talk between these proteins and the five steroid receptors increases the combinatorial complexity for regulation of development in vertebrates.

Regulation of the steroid response by selective expression of enzymes

Flexibility and complexity in vertebrate differentiation and development also arises from temporal and tissue-specific expression of enzymes that metabolize steroids [12-17]. 11β-HSD (Fig. 2) is an example of how selective expression of an enzyme is critical to the action of the mineralocorticoid aldosterone [26]. Humans and other mammals contain two distinct 11β-HSDs. 11β-HSD-type 1 is primarily a reductase, catalyzing, for example, the formation of cortisol from cortisone. 11β-HSD-type 2 is primarily an oxidase, catalyzing the inactivation of cortisol to cortisone, which is crucial for aldosterone action because the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) have similar affinity for cortisol [27]. The physiological concentration of cortisol is over 100-fold higher than aldosterone, which should prevent aldosterone from occupying the MR. How can aldosterone act in the distal tubule of the kidney to regulate transport of sodium and potassium? Selective expression in the distal tubule of the 11β-HSD-type 2 catalyzes the inactivation of cortisol to cortisone, preventing cortisol from occupying the MR. Aldosterone is inert to 11β-HSD-type 2. Thus, aldosterone is free to occupy the MR and regulate the mineralocorticoid response.

This role of selective expression of 11β-HSD-type 2 in aldosterone action illustrates how steroid-metabolizing enzymes can add to the versatility of steroid-mediated differentiation and development in vertebrates. Similar functions are found for other steroid dehydrogenases, which act at C3 and C17 to regulate the local concentrations of androgens and [8,14,15,17] estrogens.

Enzyme-mediated regulation of the steroid response arose in a protochordate and/or a primative vertebrate

Early in vertebrate evolution, when duplications of an ancestral steroid receptor led to two or more steroid receptors, these receptors likely had not diverged sufficiently in the ligand-binding domain to have high specificity for estrogens, androgens, estrogens, glucocorticoids and progestins. At that time, selective expression of enzymes that reversibly activated and inactivated steroids (Fig. 2) would be a important mechanism for controlling steroid access to a receptor, allowing steroids to influence diversification of vertebrates during the Cambrian, as well as after vertebrates colonized land [14].

To investigate the origins of enzyme-mediated regulation of estrogens and glucocorticoids, I did BLAST analysis of the genome of *Fugu rubripes* with human 11β-HSD-types 1 and 2, which are the key enzymes for regulating glucocorticoid concentrations in land vertebrates and 17β-HSD-types 1, 2, 7, which are the main enzymes that regulate the concentrations of estrogens in land vertebrates [14,15,17,28]. Orthologs of 11β-HSD-types 1 and 2 and 17β-HSD-types 1, 2, and 7 were found in *Fugu*. SINFRUP 71282 has a BLAST score of 10^{-59} with human 11β-HSD-type 1. *Fugu* SINFRUP 65346 is a close homolog of 11β-HSD-type 2 and 17β-HSD-type 2 , which are close to each other on a phylogenic tree of steroid dehydrogenases [28]. Pairwise BLASTcomparisons of Fugu SINFRUP 65346 with human 11β-HSD-type 2 and 17β-HSD-type 2 yields e-values of $2x\,10^{-81}$ and 10^{-66}, respectively. *Fugu* SINFRUP 67620 has a BLASTscore of 10^{-79} with human 17β-HSD-type 1. *Fugu* SINFRUP 71786 has a BLAST score of 10^{-121} human 17β-HSD-type 7.

Thus, it appears that bony fish contain key 11β-HSDs and 17β-HSDs that are necessary for regulation of estrogen and glucocorticoid concentrations. An ortholog to another important steroid dehydrogenase, 3β-HSD, was found in southern stingray, a cartilaginous fish, by Nunez and Trant [Genbank accession AAB63191]. 3β-HSD activity is essential for the synthesis of precursors of estrogens.

To investigate whether *Ciona* contains orthologs of enzymes that regulate estrogen and glucocorticoid action in vertebrates, I did BLAST searches of the *Ciona* genome with human and *Fugu* 11β-HSD-types 1 and 2, 17β-HSD types 1, 2 and 7 and human and stingray 3β-HSD. Two *Ciona* genes TC10140 and TC10141 contain the sequences that match human 17β-HSD-type 7. The *Ciona* gene contains a characteristic segment unique to 17β-HSD-type 7. However, there is only 42% sequence identity between TC10140 and human 17β-HSD-type 7 in 130 residues at the C-terminus, where substrate specificity arises [29], which leaves ambiguous the substrate of this enzyme. *Ciona* did not contain a gene with greater than 35% sequence identity to the other steroid dehydrogenases [data not shown]. These analyses suggest that dehydrogenases that regulate steroid hormone action evolved in a cephalochordate or a fish. However, it also is possible that these enzymes were lost in *Ciona*.

Testing of this hypothesis will come from a sequence analysis of the genomes of amphioxus, hagfish and lamprey for orthologs of receptors and enzymes that mediate the

response to adrenal and sex steroids. An amphioxus genome sequencing project is underway. Eventually either hagfish or lamprey will be sequenced due to the phylogenetic importance of these fish. If a steroid receptor ortholog is found in amphioxus, then functional analyses of the gene(s) and steroid-metabolizing enzymes will be possible. It also will be possible to study *cis*-regulatory sequences of putative ancestral steroid receptor genes as has been done with amphioxus Hox genes in transgenic mice [30]. Such studies will establish the role of steroid receptors and the enzymes that regulate steroid concentrations in differentiation and development in primitive vertebrates, resolving part of the ‘‘genome size’’ enigma.

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