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Recent Insights into the Origins of Adrenal and Sex Steroid Receptors

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Summary. The recent cloning by Thornton (Proc. Nat. Acad. Sci USA 98, 5671-5676, 2001) of an estrogen receptor, progesterone receptor and corticoid receptor from lamprey provides important insights into the early evolution of adrenal and sex steroid receptors and an opportunity to elucidate the ancient steroids that regulated gene transcription. Inclusion of lamprey sequences in a steroid receptor phylogeny indicates that the estrogen receptor is the most ancient of these receptors, followed by the progesterone receptor and the corticoid receptor. Thornton proposed that estradiol was the earliest of the steroids to activate a steroid receptor. An alternative hypothesis is that an steroid in the Δ^5 pathway activated the ancestral estrogen receptor.

Introduction.

In the last few years, the origins of adrenal and sex steroid signaling have emerged as the sequences for the genes of androgen receptor (AR), estrogen receptor (ER), glucocorticoid receptor (GR), mineralocorticoid receptor (MR) and progesterone receptor (PR) from mammals, amphibia and fish have been determined. Sequence analyses show that steroid receptors belong to the nuclear receptor family, a large and diverse family of transcription factors (Laudet 1997, Owen & Zelent 2000). The nuclear receptor superfamily also contains receptors for thyroid hormone, prostaglandins, 1,25-dihydroxy-vitamin D₃ and xenobiotics, as well as receptors with no known ligand, which are called orphan receptors (Enmark & Gustaffsson 1996).

The importance of steroid receptors and other nuclear receptors in reproduction, differentiation, development and homeostasis in vertebrates provided an early stimulus for cloning and sequencing these proteins from a variety of multicellular animals. Thus, even ten years ago, there was a sufficient number of diverse nuclear receptor sequences to allow a comprehensive phylogenetic analysis (Laudet *et al.* 1992), which showed that the adrenal and sex steroid receptors form a separate clade, distinct from other

nuclear receptors. The AR, PR, GR and MR are in one subgroup and the ER is in a separate subgroup. In the following years, sequences from amphibia and teleosts (jawed fish) became available, which further clarified early events in steroid receptor evolution. These analyses showed that the AR and PR cluster in one subgroup, and the MR and GR cluster in another subgroup (Baker 1997, Laudet 1997, Escriva *et al.* 1997). The phylogenetic tree indicated that an ancestral steroid receptor arose in a protochordate, such as a cephalochordate (e.g. amphioxus) or a urochordate (e.g. tunicate). If steroid receptors arose after the separation of deuterostomes and protostomes, then invertebrates such as *Caenorhabditis elegans* and *Drosophila*, which have nuclear receptors, would not have adrenal and sex steroid receptors (Baker 1997). Analysis of the genomes of *C. elegans* (Sluder & Maina 2001) and *Drosophila* supports this prediction (Robinson-Rechavi *et al.* 2001).

The patterns of the phylogenetic tree for steroid receptors indicate that there were two waves of gene duplications in the early evolution of steroid receptors (Baker 1997, Laudet 1997, Escriva *et al.* 1997, 2000). These duplications are proposed to be associated with genome size duplications that occurred early in the evolution of vertebrates (Holland 1999, Shimeld & Holland 2000).

The availability of a variety of nuclear receptor sequences was exploited by Escriva *et al.* (1997) to investigate when steroid receptors and other ligand-binding nuclear receptors arose. Escriva *et al.* constructed PCR primers to the highly conserved DNA binding domain on a variety of nuclear receptors, which were used to probe for their presence in deuterostomes and protostomes, including diploblasts, such as jelly fish. Escriva *et al.* found AR, ER, GR, and PR ancestors in teleosts and sharks. They found a PR ancestor in hagfish, a jawless fish. No adrenal and sex steroid receptors were found in invertebrates. Interestingly, the estrogen-related receptor (ERR), a sister group of the ER for which a ligand has not been identified, was found in a cephalochordate and in a urochordate. Escriva *et al.*'s report gave strong experimental support for the hypothesis that steroid receptors are a "recent" evolutionary innovation, arising in a deuterostome prior to or at the base of the Cambrian explosion about 540 million years ago.

However, further analysis of steroid receptor sequences from organisms that are deeper in the metazoan phylogeny is needed to clarify the origins of adrenal and sex steroid receptors. Thus, the recent report by Thornton (2001) of the cloning of estrogen, corticoid (CR), and progesterone receptors from lamprey, a jawless fish, is an important advance in understanding the origins of adrenal and sex steroid hormone signaling. The lamprey steroid receptor sequences were used by Thornton to construct a comprehensive phylogenetic tree of vertebrate steroid receptors. The branch lengths clearly show that the ER is the ancestral receptor of the adrenal and sex steroid receptor clade of the nuclear receptor family. While ERR was not shown in the phylogeny, Thornton's analysis implies that ERR also is close to the ancestral steroid receptor. Duplication of this ancestral ER and divergence of the redundant gene

led to a 3-ketosteroid receptor that most resembles the PR. Duplication of the ancestral 3-ketosteroid receptor and divergence led to the CR, which most resembles the GR.

An intriguing proposal by Thornton (2001) is that estradiol is the ancestral ligand of the ER. As Thornton notes, estradiol is the last steroid in the pathway for synthesis of steroid hormones [Figure 1]. Selection for estradiol also selects for the intermediates, such as progesterone and testosterone despite the absence of their receptors. These upstream steroids began to regulate transcription after duplication and divergence of the ancestral ER to form the PR and the subsequent descent of the AR from a duplicated PR. Thornton calls this process ligand exploitation, in which the ligand is a driving force in the functional evolution of its receptor.

Also consistent with ligand exploitation, but simpler from the viewpoint of steroid synthesis, is the hypothesis that the ancestral ER was activated by a Δ^5 -steroid that is upstream of estradiol [Figure 1] (Baker 2000). For example, Δ^5 -androstenediol has long been known to have estrogenic activity (Huggins et al. 1954) and to bind to the ER (Garcia & Rochefort 1979). Kuiper et al. (1997) showed that Δ^5 -androstenediol and 3β -androstenediol bind to recombinant rat ER α and ER β with nM affinity. They also found that androgens with 3α -alcohols have substantially lower affinity for the ER. Moreover, Miyamoto et al. (1998) showed that Δ^5 -androstenediol can regulate estrogen-responsive genes by binding to the ER. This may be “fossil” evidence for one or more ancestral steroids with a saturated A-ring, containing a 3β alcohol that activated the ancestral ER. It is not unreasonable to suppose that the ER - as well as, PR and CR - in lamprey and in primitive fish have different ligand binding specificity than their orthologs in mammals and other land vertebrates. Indeed, fish PR recognizes 17α -hydroxyprogesterins (Todo et al., 2000), unlike the PR in mammals.

The above hypothesis does not require that the ER-containing organism express a $3\beta/\Delta^{5-4}$ hydroxysteroid dehydrogenase ($3\beta/\Delta^{5-4}$ -HSD) that catalyzes the formation of Δ^4 steroids (Simard et al. 1996), which are precursors of estradiol [Figure 1]. Our recent BLAST database search (Altshul *et al.* 1997) did not find any proteins with at least 35% sequence identity to $3\beta/\Delta^{5-4}$ -HSD in *D. melanogaster*, *C. elegans* or any other invertebrate (not shown). $3\beta/\Delta^{5-4}$ -HSD is found in mammals and trout ((Sakai et al. 1994). Sequences with over 40% identity to a known $3\beta/\Delta^{5-4}$ -HSD are found in cartilaginous fish. These data are consistent with the ancestral ER arising prior to the evolution of $3\beta/\Delta^{5-4}$ -HSD that converted Δ^5 steroids to Δ^4 steroids in the pathway for estradiol synthesis. Diversification of steroid

receptors would occur with the evolution of a $3\beta/\Delta^{5-4}$ -HSD that catalyzed the conversion of pregnenolone to progesterone, providing ligands that would regulate transcriptional activity of the ancestral PR and its descendents, the CR and AR. Indeed, the early evolution of steroid hormone action depended on $3\beta/\Delta^{5-4}$ -HSD and other hydroxysteroid dehydrogenases (Baker, 2001), as well as duplications of ancestral steroid receptors.

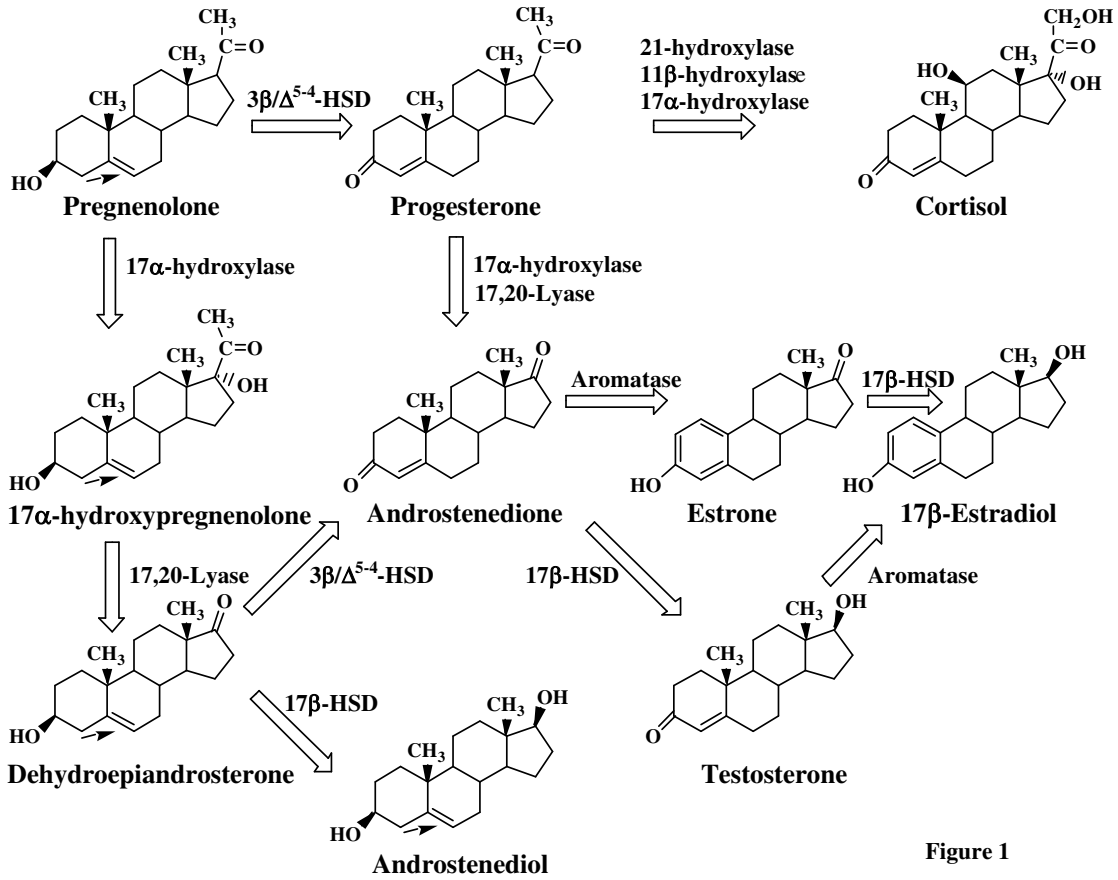


Figure 1

Figure 1. Pathway for the synthesis of adrenal and sex steroids.

Steroids in the Δ^5 pathway are formed from pregnenolone by enzymes that are also found in the Δ^4 pathway. An arrow denotes the Δ^5 position. $3\beta/\Delta^{5-4}$ hydroxysteroid dehydrogenase is the enzyme that converts Δ^5 steroids to Δ^4 steroids.

What next?

Investigation of the binding of various Δ^4 and Δ^5 steroids to lamprey ER, PR and CR will elucidate how these receptors compare with their orthologs in mammals and other land vertebrates, providing clues to the ancient ligands that regulated steroid hormone responses. In particular, analysis of the binding of estradiol and of Δ^5 steroids with 3α and 3β -alcohols to lamprey ER and the activity of these steroids in assays with lamprey ER and a reporter gene will clarify the nature of the ligand(s) that bound to the ancestral steroid receptor. Further information about the earliest steroid receptors and the origins of $3\beta/\Delta^{5-4}$ -HSD will come from the sequencing of the genomes of amphioxus, hagfish and lamprey. It also will facilitate identifying the genes that were regulated by the ancestral steroid receptors. Of special interest are *hox* genes, which are regulated by estrogens and progestins in mammals and are critical for development and differentiation, including the brain of amphioxus (Manzanas et al. 2000). When did steroids begin to regulate expression of *hox* genes? By providing tools for answering this question, the identification of steroid receptors in lamprey is bringing us to a threshold of a deeper understanding of the origins of adrenal and sex steroid action.

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