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Steroid receptor phylogeny and vertebrate origins

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Abstract Vertebrates appear about 500 million years ago in the fossil record. This is only 25–50 million years after the great explosion of multicellular invertebrate body plans in the early Cambrian. On a geological time scale, this interval is a 'blink of an eye', suggesting that the evolution of regulatory genes is likely to be important in the origins of vertebrates. Here we present evidence for a role of steroid receptors in this process based on a phylogenetic analysis suggesting that receptors for androgens, glucocorticoids, mineralocorticoids, and progesterone evolved from an ancestral steroid receptor gene by two successive duplications over a brief time that could have coincided with the origins of vertebrates. Moreover, the duplications of these steroid receptors may be additional evidence for the two duplications on a genome-scale that have been proposed to be important in the evolution of vertebrates. The two successive duplications of steroid receptor genes and their subsequent sequence divergence leading to steroid-specific receptors that regulate growth, development, reproduction and homeostasis in vertebrates may have been one of the events important in vertebrate survival after the Cambrian during global extinctions that occurred about 440 and 370 million years ago.

Keywords: Steroid receptor evolution; Vertebrate origins

1. Introduction

Among the more intriguing problems in evolution is elucidation of the events that were important in the origin of vertebrates, which have important anatomical and developmental differences from their invertebrate ancestors (Gilbert, 1994; Davidson et al., 1996). Vertebrates appear in the fossil record about 500 million years ago, about 25–50 million years after the explosive appearance of a large number of invertebrate body plans in the early Cambrian (Conway Morris, 1993; Valentine, 1994). This interval is a 'blink of the eye' in the geological record. What genetic changes account for the emergence of the ancestral vertebrate from the extraordinarily diverse body plans found in invertebrates in the late Precambrian? Part of the answer is likely to come from duplications of genes that code for regulatory proteins that control

the complex developmental processes necessary for vertebrate embryonic and postnatal development (Gilbert et al., 1996). Gene duplications are a powerful mechanism for achieving biological novelty because the second gene copy is not under constraints to maintain its original function and can accept mutations, some of which will lead to new functions that provide a selective advantage for its host (Ohno, 1970; Ohta 1989; Holland, 1992). As a result gene duplications can rapidly increase an organism's developmental complexity, a requisite for the evolution of vertebrates.

2. Genomic duplications

It appears that duplications on a scale involving most of the genome occurred during the transition from simple chordates to vertebrates (Lundin, 1993; Ohno, 1993; Holland et al., 1994; Sidow, 1996). Two lines of evidence support this hypothesis. First, *Drosophila* contains single copies of regulatory genes such as *Hox*, *MyoD*, *Notch*, *Wnt-5*, *decapentaplegic*, and *Eve*, which are present as multiple copies in vertebrates. Vertebrates have four copies of *Hox*, *MyoD* and *Notch* (Atchley et al., 1994; Holland et al., 1994; Sidow, 1996; Uyttendaele et al., 1996) and two copies of *Wnt-5*, *decapentaplegic* and *Eve* (D'Esposito et al., 1991; Sidow, 1992; Kingsley, 1994). Moreover, vertebrates have about four times as many genes as *Drosophila* (Miklos and Rubin, 1996). Together, the above supports the notion that two rounds of gene duplication on the scale of the genome occurred in one or more ancestors of vertebrates (Lundin, 1993; Ohno, 1993; Holland et al., 1994; Sidow, 1996).

Here we describe a connection between the evolution of steroid hormone receptors and the origins of vertebrates that we uncovered while doing a phylogenetic analysis of steroid receptors to elucidate the origins of steroid hormone action (Baker 1991, 1996). This phylogeny indicates that two duplications of steroid receptors occurred during the interval when vertebrates were evolving from invertebrates. The fundamental role of steroids in regulating vertebrate development, reproduction, growth and homeostasis (Evans, 1988; Gilbert, 1994; DeGroot, 1995) suggests that these steroid receptor duplications provided vertebrates a selective advantage over other organisms, which would be important in vertebrate competition with the diverse multicellular organisms present in early Cambrian as well as for vertebrate survival during global extinctions that occurred about 440 and 370 million years ago (Raup, 1994). These duplications in steroid receptor genes support the model for genome-scale duplications in the origin of the ancestral vertebrate (Lundin, 1993; Ohno, 1993; Holland et al., 1994; Sidow, 1996).

3. Steroid hormone receptors belong to the nuclear receptor family

Steroid receptors belong to a class of ligand-activated nuclear receptors that include thyroid hormone, retinoic acid, retinoid X, and 15-deoxy-$\Delta^{12,14}$prostaglandin J2 receptors (Evans, 1988; Beato, 1989; Glass, 1994; Mangelsdorf et al., 1995). These receptors regulate gene

transcription by binding to specific sites on DNA. As shown in Fig. 1, ligands with very different structures bind to nuclear receptors. In fact, nuclear receptors are an excellent example of how gene duplication and divergence can generate a protein family that responds to diverse signals to regulate a wide variety of physiological processes.

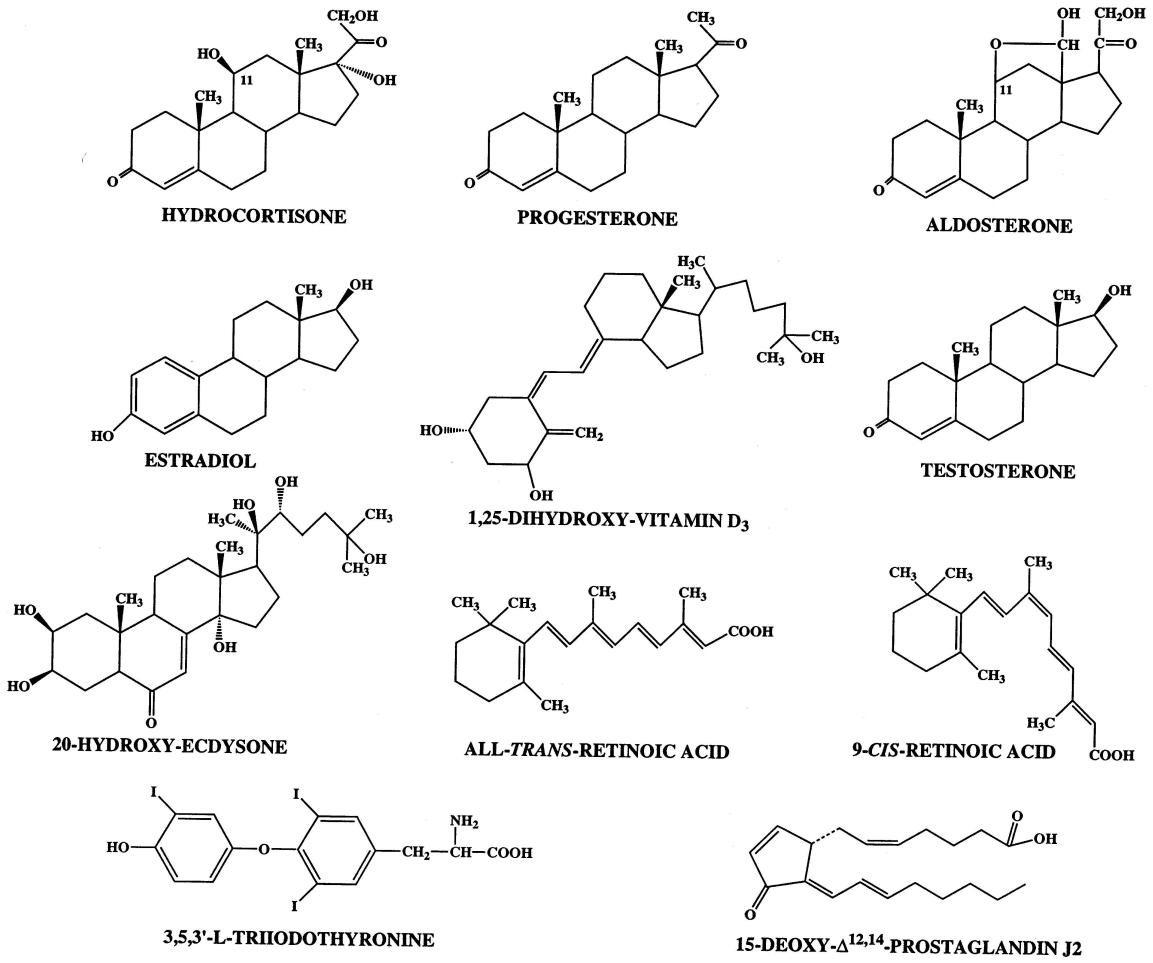

Fig. 1. Structures of steroids, retinoic acid, 15-deoxy-$\Delta^{12,14}$ prostaglandin J2 and thyroid hormone. Estradiol, testosterone, progesterone, hydrocortisone, aldosterone and 1,25-dihydroxy-vitamin D3 are active steroids in mammals. 20-hydroxy-ecdysone regulates development of insect larvae. Retinoids, thyroid hormone and 15-deoxy-$\Delta^{12,14}$ prostaglandin J2 are not steroids; they regulate gene transcription by binding to nuclear receptors that have a common ancestor with receptors for mammalian and insect steroids.

4. Steroid hormones regulate diverse physiological processes

Our focus is on a subgroup of these ligands, the steroid hormones, which have a major regulatory role in vertebrate physiology, affecting fetal and postnatal growth and development and maintaining homeostasis (Evans, 1988; Gilbert, 1994; DeGroot, 1995). Androgens and estrogens, the male and female sex hormones, respectively, regulate reproduction and sexual development. Progesterone is important in female reproduction. Glucocorticoids, such as corticosterone and hydrocortisone, regulate glucose metabolism and are part of the 'fight or

flight' response. Aldosterone regulates electrolyte balance. Moreover, this is not a complete description of the functions of these steroids. Receptors for androgens, estrogens, progesterone and mineralocorticoids are found in non-traditional sites such as the brain, where their functions are just beginning to be elucidated (Garcia-Segura et al., 1995; Funder, 1996; Wickelgren, 1997).

5. Phylogeny of steroid receptors

A phylogeny of steroid receptors and related hormone receptors based on a comparison of their hormone binding domains is shown in Fig. 2 (Fitch and Margoliash 1967; Feng and Doolittle, 1990). Table 1 shows the percentage identity of pairwise comparisons of the sequences. The general branching of the phylogenetic tree is in good agreement with that of Laudet et al. (1992) and Gronemeyer and Laudet (1995).

The data in Table 1 and Fig. 2 show that the receptors for androgens, glucocorticoids, mineralocorticoids and progesterone cluster together, with sequence identity of 50% or higher. An important point for our later analysis is that the human sequences have strong similarity to the homologs in amphibia and fish. Thus, the extensive sequence divergence of adrenal steroid and progesterone receptors from other nuclear receptors occurred before the appearance of fishes.

Next closest to the adrenal steroid and progesterone receptors is the branch with the estrogen receptors with an ancestral node at 'B', which is 36 units from 'A'. The human alpha and beta isoforms of the estrogen receptor diverged from 'b', before the separation of fishes and amphibia.

The other nuclear receptors are substantially distant from the adrenal and sex steroid receptors. The RXR receptor is closest to the estrogen receptor branch. Node 'D' marks the deepest part of the tree, and it contains an invertebrate receptor, which recognizes the steroid ecdysone. The human retinoic acid receptor and thyroid hormone receptor clustering is in agreement with other analyses (Laudet et al., 1992; Gronemeyer and Laudet, 1995).

The lengths of the branches are proportional to the genetic distances, providing an insight into the evolution of these members of nuclear receptor family, if most of the changes can be described by a molecular clock; that is, if the mutations accumulate linearly with time. This assumption has proven reasonable for analyses of sequence changes over the last billion years (Doolittle et al., 1996; Wray et al., 1996). It appears to be valid for the overall evolution of the human nuclear receptors, which are about the same distance from the deepest branch (Fig. 2). Duplications in the deepest branch led to receptors that recognize very different hormones (Fig. 1) that regulate development of multicellular animals. The gene duplication at 'B' led to the separation of the estrogen receptor from the four other steroid receptors, which then separated from each other with a duplication at 'A' followed by duplications at 'a'. The alpha and beta isoforms of the estrogen receptor diverged at 'b'. It is duplications at 'A', 'a' and 'b' that we propose coincided with genomic duplications that were important in the origins of vertebrates.

Table 1
Identical (%) amino acids in the hormone binding domain of nuclear receptors of the phylogeny shown in Fig. 2

	HAR	XAR	HPR	HMR	XMR	HGR	XGR	TGR	HERα	XERα	TERα	HERβ	HRXR	HRAR	HTR	ECDY	PPRA
HAR	—																
XAR	87.8	—															
HPR	54	54	—														
HMR	50.8	50.8	55.1	—													
XMR	50	51.6	54.7	84.7	—												
HGR	49	47.8	53.8	56.1	55	—											
XGR	49.8	49.4	53.8	57.7	54.6	76.3	—										
TGR	50.6	49.8	56.5	58.1	57.3	71.5	70	—									
HERα	19.6	20.8	22.8	23.2	23.2	25.7	23.7	26.1	—								
XERα	19.6	21.2	23.2	21.2	21.6	23.3	23.3	23.7	79	—							
TERα	20.4	22	22.8	22.4	22.4	21.7	21.3	23.7	60	55.3	—						
HERβ	22.5	23.7	24.1	24.5	24.5	24.6	25.8	25.4	54.9	52.5	53.3	—					
HRXR	20.3	19.8	19.4	19.8	19.8	20.4	20.8	19	29.1	27.5	29.6	26.6	—				
HRAR	16.4	17.7	17.7	20.3	17.2	16.8	17.2	16.8	20.4	19.8	18.7	19	25.6	—			
HTR	15.9	16.3	15	19	19.8	14.2	15	15	17.3	17.3	15.5	18.6	21.2	31	—		
ECDY	16.7	18.4	19.7	21	21.8	21.9	22.8	20.6	19.5	20.3	20.4	18.9	24.1	24.6	25.5	—	
PPAR	14.9	15.3	13.1	16.2	16.6	15.8	14.5	15.4	15.4	14.7	17.5	14.5	21.3	23	24.3	20.3	—
HVDR	14	15.3	15.3	17.9	18.7	15.8	16.7	14.1	20.7	20.3	20.7	18.5	18	22.5	21.7	25.4	23

HAR, human androgen receptor; XAR, *Xenopus laevis* androgen receptor; HPR, human progesterone receptor; HMR, human mineralocorticoid receptor; XMR, *Xenopus laevis* mineralocorticoid receptor; HGR, human glucocorticoid receptor; XGR, *Xenopus laevis* glucocorticoid receptor; TGR, trout glucocorticoid receptor; HERα, human estrogen receptor-α; XERα, *Xenopus laevis* estrogen receptor-α; TERα, trout estrogen receptor-α; HERβ, human estrogen receptor-β; HRXR, human retinoid X receptor-α; HRAR, human retinoic acid receptor-α1; HTR, human thyroid hormone receptor-α1; ECDY, *Drosophila* ecydsone receptor; PPAP, human peroxisome proliferator activated receptor-γ; HVDR, human 1,25-$(OH)_2$vitamin D_3 receptor.

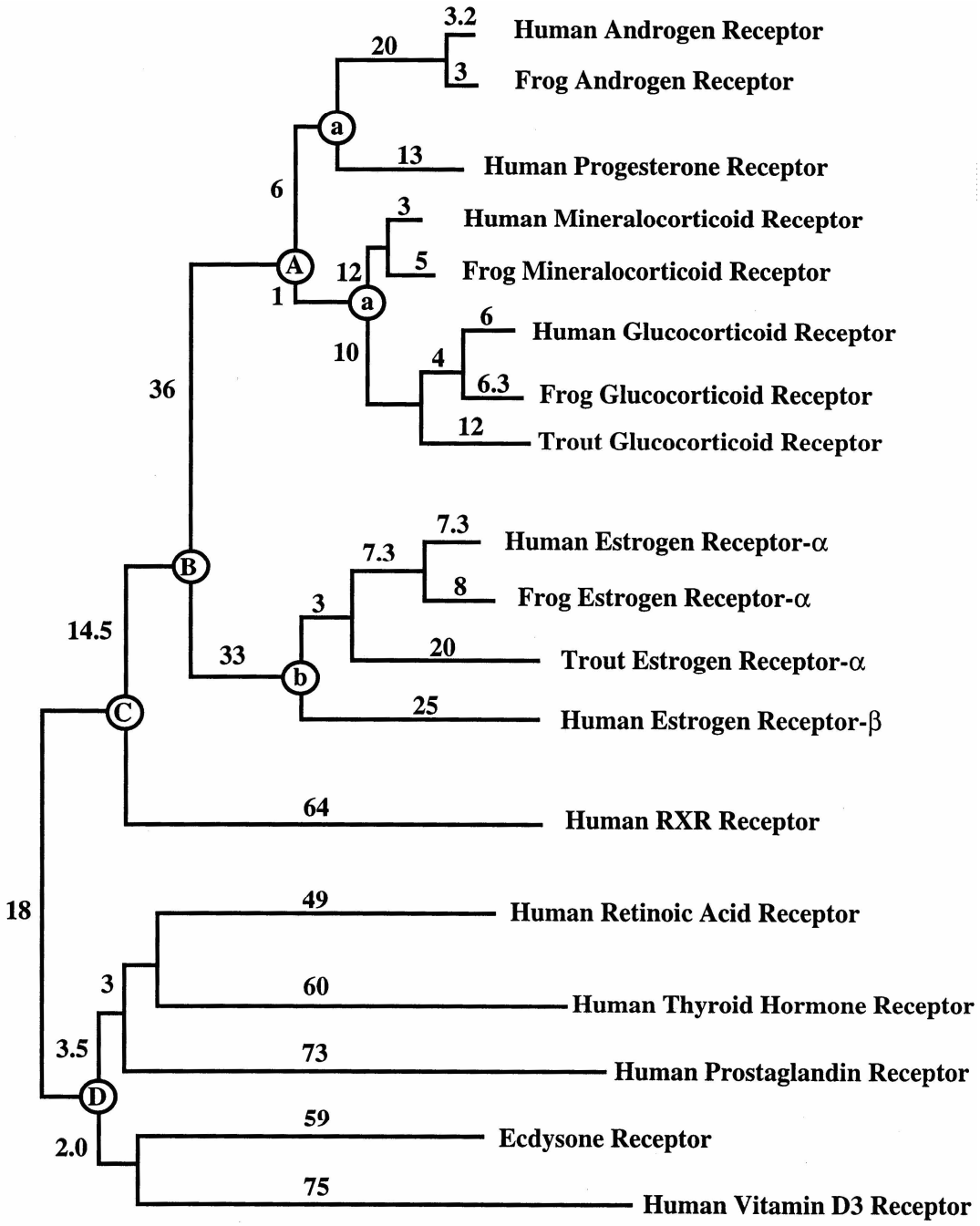

Fig. 2. Evolution of receptors for steroids, thyroid hormone, 15-deoxy-$\Delta^{12,14}$ prostaglandin J2 and retinoids. A phylogenetic tree based on the hormone binding domains of nuclear receptors was constructed with the algorithm of Feng and Doolittle (1990). In this method, first the protein sequences are progressively aligned using the Dayhoff PAM-250 scoring matrix to assess pairwise similarity of each sequence with the others; the scores are assembled into a distance matrix. The method of Fitch and Margoliash (1967) is used to obtain branching order for the sequences. Branch lengths are calculated by linear regression analysis of the best fit of pairwise distances and branching order. Major nodes for separation of steroid receptors from other nuclear receptors are denoted by 'A', 'B', 'C' and 'D'. The lengths of the branches are proportional to the relative distance between the sequences. For example, human progesterone and androgen receptors are 13 and 23.2 units distant, respectively, from their common ancestor.

6. Slow evolution of steroid receptors in vertebrates

Comparisons of the evolution of orthologous steroid receptors indicate slow sequence divergence in land animals. Four human steroid receptors have about 75–88% sequence identity to their *X. laevi*s orthologs (Table 1). The fish estrogen and glucocorticoid receptors have about 70 and 60% sequence identity, respectively, to their human orthologs. This puts these steroid receptors in the class of slowly changing proteins duplications. (Doolittle, 1992). As a benchmark comparison, the fish and human hemoglobin A have about 45% sequence identity.

A striking feature of the phylogeny is that the distances of the branches leading to the androgen, glucocorticoid, mineralocorticoid and progesterone receptors are very short compared with their distances from the other hormone receptors. This indicates that these four steroid receptors evolved by two closely spaced gene duplications.

7. When did these duplications occur?

Thus far, receptors for androgens, estrogens, glucocorticoids, mineralocorticoids and progesterone receptors have been found only in vertebrates. The yeast genome, which is completely sequenced does not have a gene with strong sequence similarity to a nuclear receptor. Nuclear receptors are found in *Caenorhabditis elegans* and *Drosophila*. The *C. elegans* genome, which is about 72% sequenced, has a very distantly related retinoic acid receptor homolog; *Drosophila* has a retinoid X receptor homolog and an ecdysone receptor. The complete sequencing of these genomes will clarify substantially the origins of adrenal and sex steroid receptors.

The analysis in Fig. 2 predicts that adrenal and sex steroid receptors arose after the separation of *Drosophila*. Thus, neither *Drosophil*a nor *C. elegan*s should have a close homolog of a vertebrate adrenal or sex steroid receptor. The latter genome should be completely sequenced in about six months, providing a test of this hypothesis. We predict that the ancestral steroid receptor will be in a tunicate or *Amphioxus*, a primitive chordate; that is, in an organism with a genome 1/4 the size of vertebrate genomes. The phylogenetic analysis predicts that the ancestral receptor will most resemble the estrogen receptor. However, its function may be different from a reproductive one as in fish and mammals. Indeed, the ancestral receptor may regulate development of the nervous system. Some of the actions of estrogens in the brain (Garcia-Segura et al., 1995; Wickelgren, 1997) may have originated in the early evolution of vertebrates from primitive chordates. Sequencing of the *Amphioxu*s genome should clarify the origins of adrenal and sex steroid receptors and permit knockout experiments to elucidate the functions of the ancestral steroid receptor(s).

Hox genes have proven to be one of the most important markers for the evolution of vertebrates, which are characterized as containing four clusters of the *Hox* gene complex. The presence in Amphioxus of just one *Hox* gene complex indicates that the two rounds of genomic scale duplications occurred after *Amphioxus* diverged from its tunicate ancestor (Holland et al.,

1994; Sidow, 1996). The analysis presented in this paper suggests that adrenal and sex steroid genes may be another useful probe for elucidating the origins of vertebrates. And while *Hox* gene expression clearly is important in the evolution of the body plan of early vertebrates, ancestral steroid receptors may also be important in this process.

The physiological responses mediated by adrenal and sex steroid receptors (Evans, 1988; Gilbert, 1994; DeGroot, 1995; Garcia-Segura et al., 1995; Funder, 1996; Wickelgren, 1997) provided early vertebrates with an advantage in competing with the diverse organisms that evolved during the Cambrian explosion and lacked either some or all of these steroid receptors. Indeed, the responses to environmental disturbances that are mediated by adrenal and sex steroids may have been important in the survival of vertebrates during later global catastrophes, two of which occurred 440 and 370 million years ago (Raup, 1994).

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