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Evolution of 17β-Hydroxysteroid Dehydrogenases and Their Role in Androgen, Estrogen and Retinoid Action

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Abstract. 17β-hydroxysteroid dehydrogenases (17β-HSDs) regulate androgen and estrogen concentrations in mammals. By 1995, four distinct enzymes with 17β-HSD activity had been identified: 17β-HSD-types 1 and 3, which *in vivo* are NADPH-dependent reductases; 17β-HSD-types 2 and 4, which *in vivo* are NAD^+-dependent oxidases. Since then six additional enzymes with 17β-HSD activity have been isolated from mammals. With the exception of 17β-HSD–type 5, which belongs to the aldoketo-reductase (AKR) family, these 17β-HSDs belong to the short chain dehydrogenases/reductases (SDR) family. Several 17β-HSDs appear to be examples of convergent evolution. That is, 17β-HSD activity arose several times from different ancestors. Some 17β-HSDs share a common ancestor with retinoid oxido-reductases and have retinol dehydrogenase activity. 17β-HSD-types 2, 6 and 9 appear to have diverged from ancestral retinoid dehydrogenases early in the evolution of deuterostomes during the Cambrian, about 540 million years ago. This coincided with the origin of nuclear receptors for androgens and estrogens suggesting that expression of 17β-HSDs had an important role in the early evolution of the physiological response to androgens and estrogens.

1. Introduction

There is substantial interest in the physiological effects of androgens and estrogens because these steroids regulate reproduction and development in mammals and other vertebrates (Baulieu and Kelly, 1990), and also because many cancers require either androgens or estrogens for growth (Feigelson and Henderson, 1996; Aquilina et al., 1997). For a long time, the androgen receptor (AR) and estrogen receptor (ER) were the major research focus because these nuclear receptors mediate the actions of these steroids. It is only in the last decade that the importance of 17β-hydroxysteroid dehydrogenase (17β-HSD) in regulating androgen and estrogen action has become appreciated (recent review in Peltoketo et al., 1999). Oxidation of the C17 alcohol either on dihydrotestosterone, testosterone or 17β-estradiol to a ketone by 17β-HSD reduces the potency of these steroids [Figure 1]. Similarly, reduction of the C17 ketone on 5α-androstanedione, androstenedione, or estrone yields the biologically active steroid. Thus, the presence or absence of 17β-HSD in cells is an effective method for regulating the access of active androgen or estrogen to steroid-responsive cells.

By 1995, four distinct 17β-HSDs had been isolated (Andersson, 1995; Baker, 1996; Peltoketo et al., 1999). *In vivo*, 17β-HSD-types 1 and 3 catalyze the synthesis of active steroids with NADPH as the coenzyme. *In vivo*, 17β-HSD-types 2 and 4 catalyze the inactivation of active steroids with NAD^+ as the coenzyme.

The four 17β-HSDs belong to the short chain dehydrogenases/reductases (SDR) family (Jornvall et al., 1995), which consists of a large and diverse group of oxido-reductases that are found in bacteria, plants and animals (Baker, 1991; Jornvall et al., 1995; Bailey et al., 1996). Some SDRs are involved in the synthesis and degradation of a variety of intercellular signals. This includes nod factors, which are signals secreted by rhizobia, soil bacteria that form nitrogen-fixing nodules in the roots of legumes (Baker, 1991), as well as steroids and prostaglandins, which regulate growth, development and homeostasis in mammals and other animals (Baker, 1991).

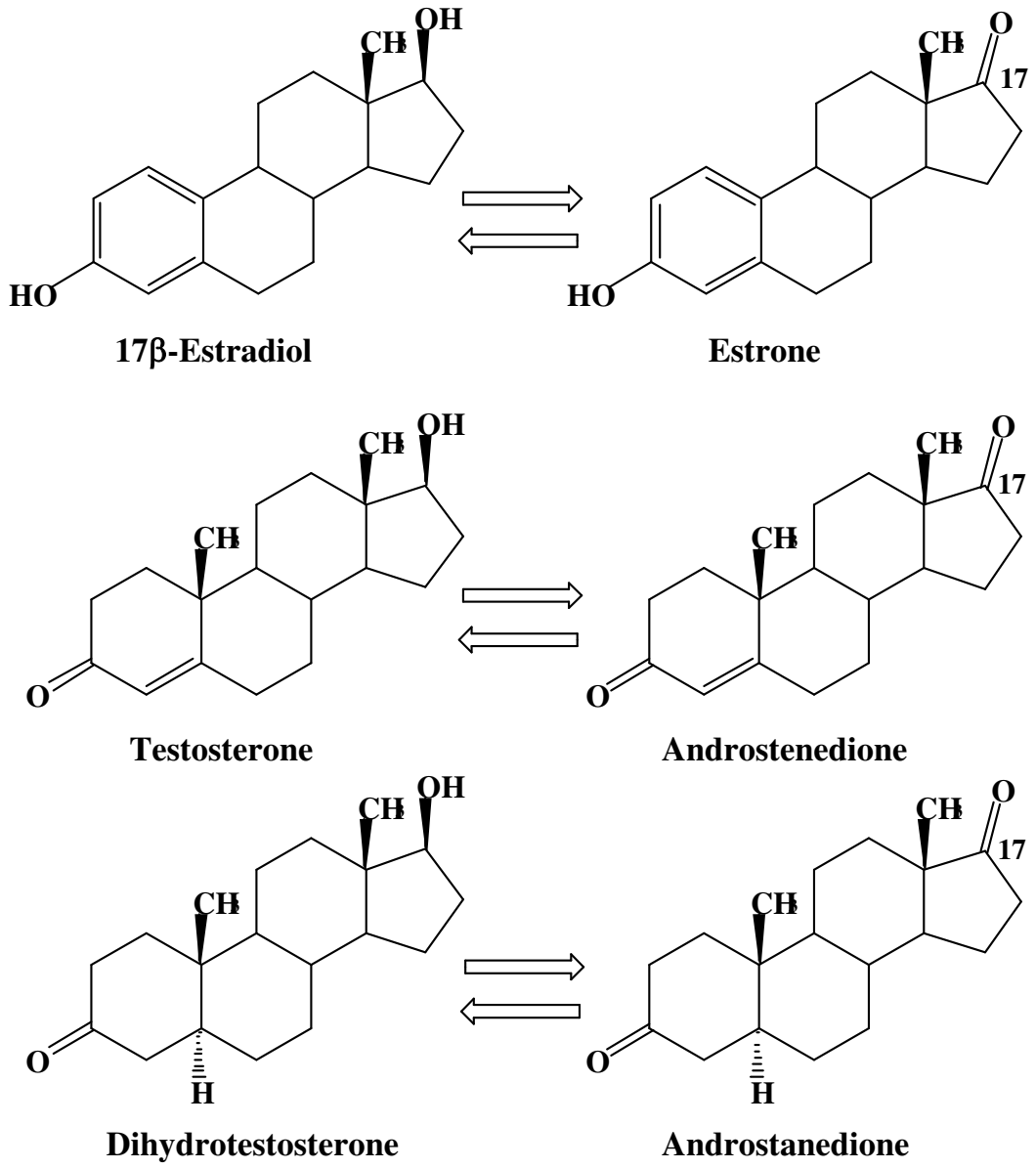

Figure 1. **Reactions catalyzed by 17β-hydroxysteroid dehydrogenases. Oxidation of the C17 alcohol on steroids yields an inactive or less active steroid.**

Although the four 17β-HSDs belong to the same protein superfamily, their amino acid sequences have less than 25% identity to each other. Either these sequences diverged a long time ago from an ancestral 17β-HSD or that there was convergence in the evolution of 17β-HSD activity (Baker, 1996). That is, 17β-HSD activity arose independently from more than one ancestral enzyme that did not have 17β-HSD activity.

Since 1995, the number of enzymes with 17β-HSD has expanded as newly sequenced genes were examined for metabolism of estrogens and androgens. At the time of this paper, at least 10 different enzymes have been identified as 17β-HSDs (for properties of types 1-8 see Fomitcheva et al., 1998; Peltoketo et al., 1999). Nine are SDRs. One, 17β-HSD-type 5, belongs to the aldoketo-reductase (AKR) family. 17β-HSD-type 6 (Biswas and Russell, 1997) and 17β-HSD-type 9 (Su et al., 1999) also recognize retinoids. The evolution of the 17β-HSDs that belong to the SDR family is the subject of this paper. Before analyzing the evolution of 17β-HSDs, I briefly review some principles of evolutionary analyses that will be useful in describing the complex evolution of 17β-HSD, which provides examples of several evolutionary principles.

2. Evolution

Dobzhansky (1973) eloquently summarized the importance of evolutionary analyses in modern biology: "Nothing in biology makes sense except in the light of evolution." Indeed, with the explosive growth of sequences in GenBank, including collections of genes for entire genomes in bacteria, yeast, *Caenorhabditis elegans* and *Drosophila melanogaster*, and the soon to be sequenced human genome, evolutionary analyses are increasingly used to understand biological processes. Such analyses can elucidate when specific mechanisms arose and provide clues for treating diseases. For example, what is the relationship of 17β-HSDs to retinol dehydrogenases?

Gene duplication and divergence. A fundamental mechanism for the emergence of proteins with new functions is gene duplication and divergence. An organism with two or more copies of a single gene can maintain the original function in one copy, while the other gene(s) mutate. Most mutations are deleterious, leading to reduced function or inactivation of the gene product. Indeed, the normal rate of random mutations in genes provides a window of about 10 million years for a gene to acquire a beneficial function that increases the fitness of its host organism.

Orthologs and paralogs. Closely related genes in different species can be either orthologs or paralogs. Genes are orthologs when they have diverged by speciation. For example, the hemoglobin α-chain in humans, birds and frogs are orthologs, as are human and fish estrogen receptor-α (ER-α). Paralogs are genes that diverged by gene duplication and divergence. Human hemoglobin α and β chains are paralogs, as are human ER-α and ER-β.

Convergent evolution. A classic example of convergent evolution at the level of the organism is the independent evolution of wings in bats and birds. On a molecular level, there are

several examples of convergent evolution in steroid hormone biology. Proteins with high affinity for estrogen have been "invented" at least three times in the estrogen receptor, rat α-fetoprotein and human sex hormone binding globulin from different ancestral proteins [Figure 2]. 17β-HSD-types 3 and 5 are an example of convergence. Moreover, as will be seen later, there are other examples of convergent evolution of 17β-HSD activity.

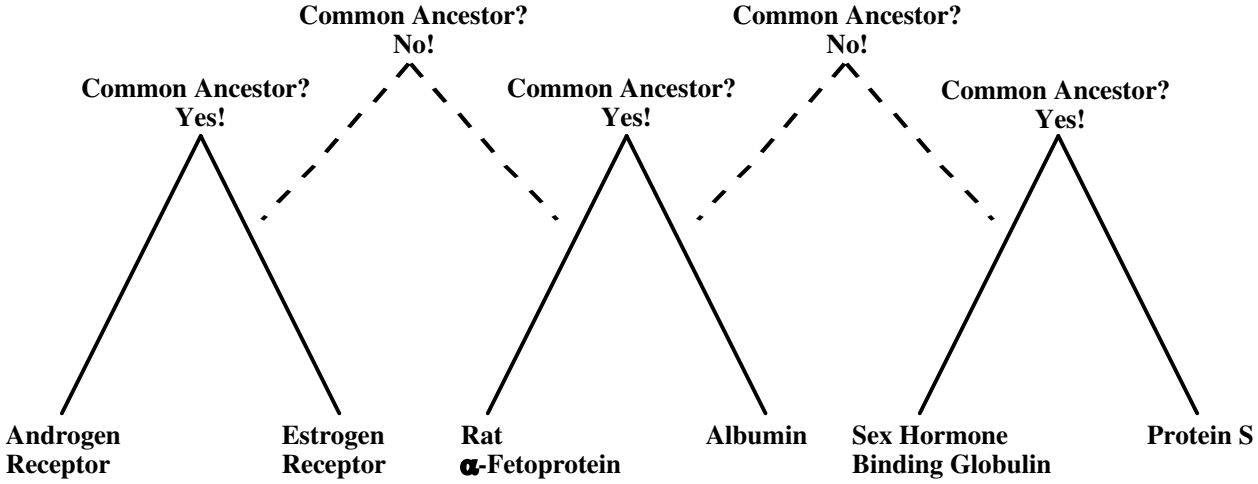

Figure 2. Convergent evolution of estrogen binding proteins.
Estrogen binding to the estrogen receptor, rat α-fetoprotein and sex hormone binding globulin is an example of convergent evolution. These estrogen-binding proteins are not homologous to each other. The estrogen receptor is homologous to nuclear receptors. Rat α-fetoprotein is homologous to albumin. Sex hormone-binding globulin is homologous to protein S.

3. When did androgen and estrogen receptors arise?

During the Cambrian period, from 545 to 530 million years ago, there was an explosive increase in the number and diversity of animals in the fossil record. Indeed, over 30 different body plans are first seen at this time. Although, the causes of this radiation are not fully understood, an increase in atmospheric oxygen, which could support the increased metabolism of larger animals, was important. It appears that the AR, ER and other steroid receptors arose just before or during the early Cambrian (Baker, 1997; Laudet, 1997).

Androgen and estrogen receptors belong to the nuclear receptor family, a large and diverse family of transcription factors. Their DNA-binding domain consists of two zinc fingers in a characteristic cysteine motif. The ligand-binding domain is at the carboxyl terminus. The sequencing of entire genomes provides information about when nuclear receptors arose as regulators of gene transcription. For example, the *Saccharomyces cerevisiae* genome does not contain nuclear receptors, which suggests that nuclear receptors did not arise in a unicellular

organism. Further support for this hypothesis will come from the soon to be completed sequences of other yeast genomes. Sufficient *Arabidopsis* genome has been sequenced to show that this plant lacks nuclear receptors. Thus, it appears that nuclear receptors arose in multicellular animals.

Escriva et al. (1997) used the DNA-binding domain of steroid receptors as probes for a PCR-based search for homologs in vertebrates and invertebrates. There is no evidence for either the ER or AR in invertebrates. The earliest evidence for ER and AR is in sharks. Phylogenetic analysis of steroid receptors and other nuclear receptors that bind ligands - vitamin D, thyroid hormone, retinoids, and ecdysone - indicates that the AR and ER arose in the Cambrian when primitive vertebrates evolved (Baker, 1997; Escriva et al., 1997). However, PCR and phylogenetic analyses indicate that nuclear receptors that bind retinoids arose in invertebrates.

4. Phylogenetic analysis of 17β-HSDs, 11β-HSDs and retinoid oxido-reductases

To investigate the relationship of the nine 17β-HSDs that belong to the SDR family, we constructed a phylogenetic tree of the core 250 amino acid segment that contains the domains necessary for catalytic activity (Jornvall et al., 1995; Bailey et al., 1996; Grundy et al., 1997). We used the Feng-Doolittle (1990) and the Fitch and Margoliash (1967) algorithms to construct this tree. We also added human 11β-HSD-types 1 and 2, human all-*trans*-retinol dehydrogenase, human retinal reductase and *Bacillus subtilis* 3-oxoacyl-acyl-carrier protein reductase to this phylogeny to investigate their relationship to 17β-HSD activity.

The phylogeny of nine 17β-HSDs is shown in Figure 3. One striking feature is the close relationship of 17β-HSD types 2, and 9 with all-*trans*-retinol dehydrogenase and 11β-HSD type 2. This relationship is confirmed by functional studies (Biswas and Russell, 1997; Su et al., 1999; Rattner et al., 2000). In particular, 17β-HSD type 9 has retinol dehydrogenase activity, as well as 3α-HSD activity (Su et al., 1999). This provides additional support for an earlier hypothesis that 17β-HSD types 2 and 6 and 11β-HSD type 2 are descended from an ancestral retinoid dehydrogenase in invertebrates (Baker, 1998a). One implication of the close relationship of 17β-HSD, 11β-HSD type 2 and retinol dehydrogenase is that dietary retinoids may affect androgen, estrogen or glucocorticoid levels by binding to hydroxysteroid dehydrogenases (Baker, 1998b).

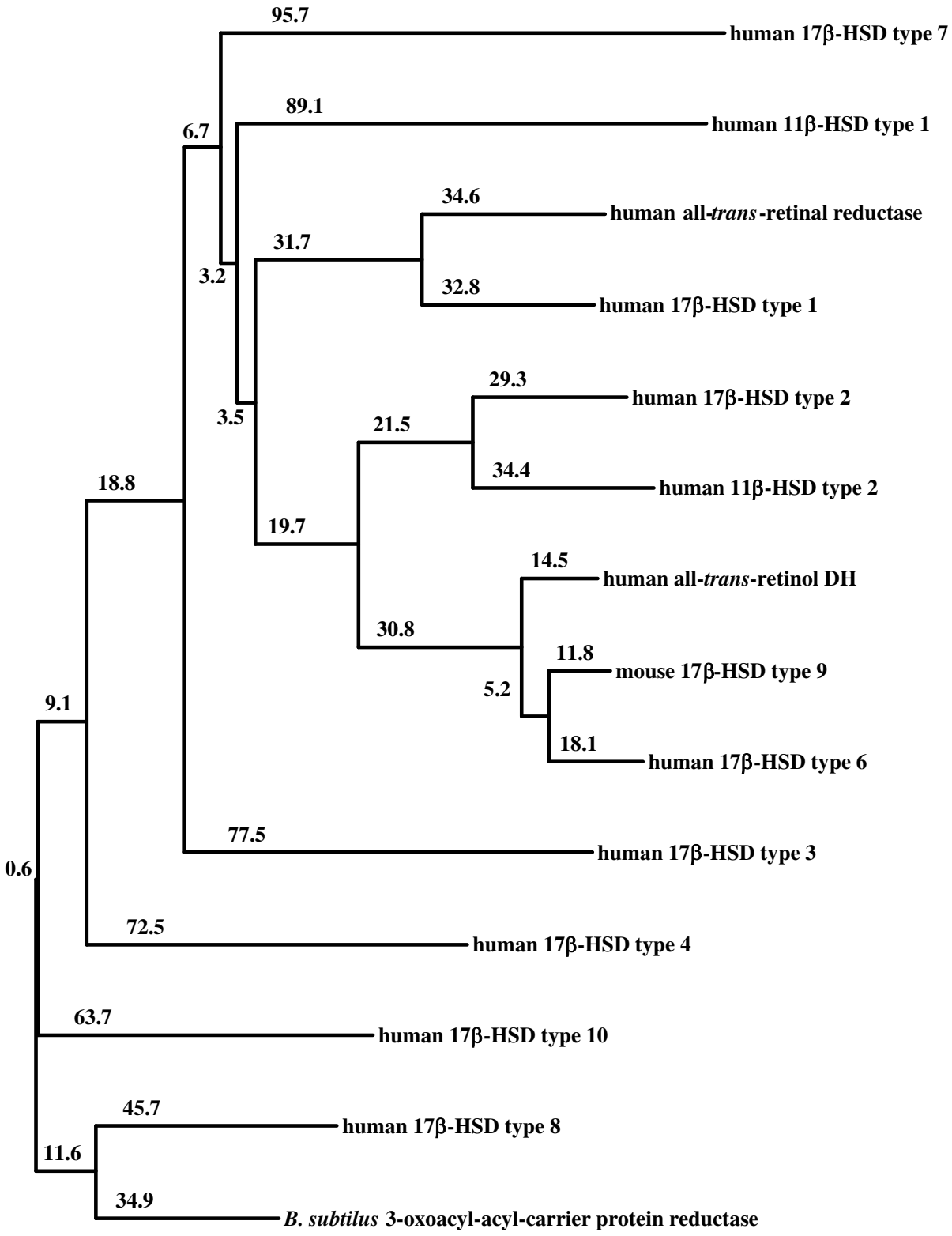

Figure 3. Phylogenetic analysis of 17β-hydroxysteroid dehydrogenases, 11β-hydroxysteroid dehydrogenases and retinoid oxido-reductases.

Feng-Doolittle (1990) algorithm was used to construct a phylogenetic tree of nine 17β-hydroxysteroid dehydrogenases, human 11β-hydroxysteroid dehydrogenase-type 2, human retinol dehydrogenase, yeast multifunctional enzyme and *B. subtilus* 3-oxoacyl-acyl carrier protein reductase. First the proteins are progressively aligned using the Dayhoff PAM-250 scoring matrix to assess their pairwise similarity with the others; the scores are assembled into a distance matrix. Then Fitch and Margoliash (1967) is used to obtain the best branching order. Branch lengths are calculated by linear regression analysis of the best fit of pairwise distances and branching order. Lengths of the branches are proportional to relative distances between sequences.

Recently, Rattner et al. (2000) reported that human all-*trans*-retinal reductase (historically named retinol dehydrogenase) in the photoreceptor outer segments has about 48% sequence identity to human 17β-HSD-type 1. GenBank does not contain a close ancestor of either enzyme in either *C. elegans* or *D. melanogaster*. Thus, the ancestry of 17β-HSD-type 1 and all-*trans*-retinal reductase remains to be elucidated. Nevertheless, the sequence similarity is sufficient to suggest determining if 17β-HSD-type 1 recognizes retinoids and if all-*trans*-retinal reductase recognizes estrogens or androgens.

D. melanogaster contains a protein with 44% identity to 17β-HSD-type 3 over a 236 amino acid segment. This suggests that human 17β-HSD-type 3 arose by a duplication of an ancestral dehydrogenase in invertebrates. The substrate for the ancestral receptor is not known. The low sequence similarity between 17β-HSD-types 1, 2 and 3 is reflected in the branch lengths in Figure 3, and it suggests that their 17β-HSD activity arose from different ancestors.

17β-HSD-types 4 and 10 have D-3-hydroxyacyl CoA dehydrogenase and L-3-hydroxyacyl CoA dehydrogenase activity, respectively. These activities arose in yeast and bacteria, before the evolution nuclear receptors. The steroid-metabolizing activity of 17β-HSD types 4 and 10 is the more recent activity and is an example of convergent evolution from ancestors that metabolized fatty acids. The Kms of 17β-HSD-types 4 (de Launoit and Adamski, 1999) and 10 (He et al., 2000) for estradiol are higher than those of other 17β-HSDs. Thus, it is not clear that 17β-HSD-types 4 and 10 have a physiological role in metabolizing steroids.

17β-HSD-type 10 has about 65% identity to homologs in *C. elegans* and *D. melanogaster* (data not shown). This indicates that there was a substantial reduction in the change of the 17β-HSD-type 10 sequence since vertebrates and invertebrates diverged. 17β-HSD-type 10 is noteworthy for its binding to β-amyloid protein (Yan et al., 1997), which has homologs in *C.*

elegans and *D. melanogaster*. The slow change in sequence of 17β-HSD-type 10 may be due to its interaction with β-amyloid protein homologs in invertebrates. If this is true, then the interaction between 17β-HSD-type 10 and β-amyloid protein arose before the separation of protostomes and deuterostomes. And this suggests that studies in *C. elegans* and *D. melanogaster* could elucidate the role of 17β-HSD-type 10 in Alzheimer's disease.

17β-HSD-type 8 has about 50% identity with proteins in *C. elegans*, which indicates that there was a slowdown in the change in the sequence of this dehydrogenase in the last 550 to 600 million years. The source of the conservation of the 17β-HSD-type 8 sequence is not understood. It may be due to interaction of 17β-HSD-type 8 with another protein, as is found for 17β-HSD-type 10. Or 17β-HSD-type 8 may have more than one catalytic activity, perhaps to metabolize a fatty acid, as well as steroids.

17β-HSD-type 7 does not show any strong sequence similarity to nonmammalian proteins, including proteins in the genomes of *C. elegans* and *D. melanogaster*. Mammalian 17β-HSD-type 7 may have diverged substantially from an invertebrate ancestor or the origin of 17β-HSD-type 7 may be in a deuterostome.

The analysis presented here indicates that 17β-HSDs have a complex and interesting evolutionary pedigree. Some 17β-HSDs arose by gene duplications of an ancestral retinoid oxido-reductase, followed by sequence divergence, leading to steroid oxido-reductase activity and preference for C11 or C17 substituents on steroids. However, selective pressures also led to convergent evolution of 17β-HSD activity from more than one ancestral oxido-reductase. Thus both divergent and convergent evolution have been important in the hydroxysteroid dehydrogenase mechanism for regulating the response to steroid hormones.

Sequence analysis indicates that some 11β- and 17β-hydroxysteroid dehydrogenases evolved from ancestral oxido-reductases at a time that is close to that for the evolution of steroid receptors in primitive vertebrates (Baker, 1997; Laudet, 1997). At that time, it is likely that both hydroxysteroid dehydrogenases and steroid receptors were important co-regulators of the response to androgens, estrogens and other steroids.

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