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Evolution of Mammalian 11β- and 17β-Hydroxysteroid Dehydrogenases-type 2 and Retinol Dehydrogenases from Ancestors in *Caenorhabditis elegans* and Evidence for Horizontal Transfer of a Eukaryote Dehydrogenase to *E. coli*

Michael E. Baker*

Department of Medicine, 0623B, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0623, U.S.A.

Physiological responses due to steroid hormones and retinoids are regulated by their cognate receptors and dehydrogenases. The origins of either regulatory mechanism are not fully understood. Here we examine the origins of the human 11β-hydroxysteroid dehydrogenase-type 2, which regulates access of glucocorticoids to cells, and 17β-hydroxysteroid dehydrogenase-type 2, which regulates access of androgens and estrogens to cells. Sequence comparisons trace their ancestry to homologs in *Caenorhabditis elegans*. These *C. elegans* proteins most closely resemble mammalian all-*trans* and 11-*cis*-retinol dehydrogenases. The similarity is sufficient ~37% to 43% identity to suggest that one or more of the *C. elegans* homologs metabolizes a retinoid. Receptors for retinoids, but not for androgens, estrogens or glucocorticoids have been identified in *C. elegans*, suggesting that retinoid-mediated gene transcription is more ancient than that for adrenal and sex steroids. We propose that the hydroxysteroid dehydrogenase-type 2 mechanism for regulating the androgen, estrogen and glucocorticoid concentrations in mammals descended from that for regulating retinoid concentrations. Interestingly, *E. coli* contains a protein with strong sequence similarity to mammalian retinol dehydrogenases. Sequence comparisons and phylogenetic analysis indicate that the *E. coli* protein may be an example of horizontal transfer from a eukaryote ancestor. © **1998 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

Steroid hormones regulate a wide variety of endocrine responses that include reproduction, development, response to stress, and electrolyte balance [1–4]. Steroids exert their biological effects by binding to a specific receptor in target cells. Upon binding the steroid, the receptor undergoes a conformational change rendering it capable of regulating the transcription of genes that evoke a physiological response that is characteristic of the steroid [3–6].

For a long time, it was assumed that the presence or absence of the receptor in a cell was the primary mechanism for regulating the activity of a particular steroid hormone. In the last decade, the role of tissue-specific 11β-hydroxysteroid dehydrogenases (11β-HSDs) and 17β-hydroxysteroid dehydrogenases (17β-HSDs) that control the concentration of active steroid in target cells has been recognized as another important regulatory mechanism [7–12]. There are two distinct 11β-HSDs (types 1 and 2), which have less than 25% sequence identity to each other, and six distinct 17β-HSDs (types 1–6) also with less than 25% sequence identity to each other.

*Correspondence to M. E. Baker. Tel: 534 4164; Fax: 534 1424; e-mail: mbaker@ucsd.edu.

It is the type-2 enzymes that are the subject of this paper. These enzymes catalyze the oxidation of alcohols at C-17 on androgens and estrogens and at C-11 on glucocorticoids, which results in steroids that are either less active or inactive (Fig. 1). By acting as gatekeepers for the access of biologically active steroids to cells, the type-2 enzymes as well as steroid receptors control the actions of androgens, estrogens, glucocorticoids and mineralocorticoids. As such, the 11β-HSD-2 and 17β-HSD-2 regulate reproduction, fetal and postnatal development, response to stress, and electrolyte balance and blood pressure.

Interference with the activity of either type-2 hydroxysteroid dehydrogenase can have devastating physiological consequences. In fact, a genetic disease, apparent mineralocorticoid excess, which is caused by mutations in 11β-HSD-2, was an important stimulus for uncovering the role of hydroxysteroid dehydrogenases in steroid action [13–15].

Our understanding of origins of 11β-HSD-2 and 17β-HSD-2 as regulators of steroid hormone action is incomplete. 11β-HSD-2 and 17β-HSD-2 are closely related — their amino acid sequences are about 45% identical. These hydroxysteroid dehydrogenases are homologous to all-*trans*-retinol dehydrogenase, 11-*cis*-retinol dehydrogenase and β-hydroxybutyrate dehydrogenase (β-OH-BDH). 11β-HSD-2 and 17β-HSD-2 have about 35% sequence identity to all-*trans*-retinol dehydrogenase [16, 17] and 30% sequence identity to 11-*cis*-retinol dehydrogenase [16, 18]. All-*trans*- and 11-*cis*-retinol dehydrogenases are about 40% and 35% identical, respectively, to β-OH-BDH [16]. This close sequence similarity is reflected in their evolution, which finds them on a branch separate from other hydroxysteroid dehydrogenases and other short-chain alcohol dehydrogenases [16]. Recently, Biswas and Russell showed that all-*trans*-retinol dehydrogenase has both 3α and 17β-hydroxysteroid dehydrogenase activity [19]. They also cloned 17β-HSD-6, which is 65% identical to all-*trans*-retinol dehydrogenase and found that retinoids inhibit oxidation of steroids

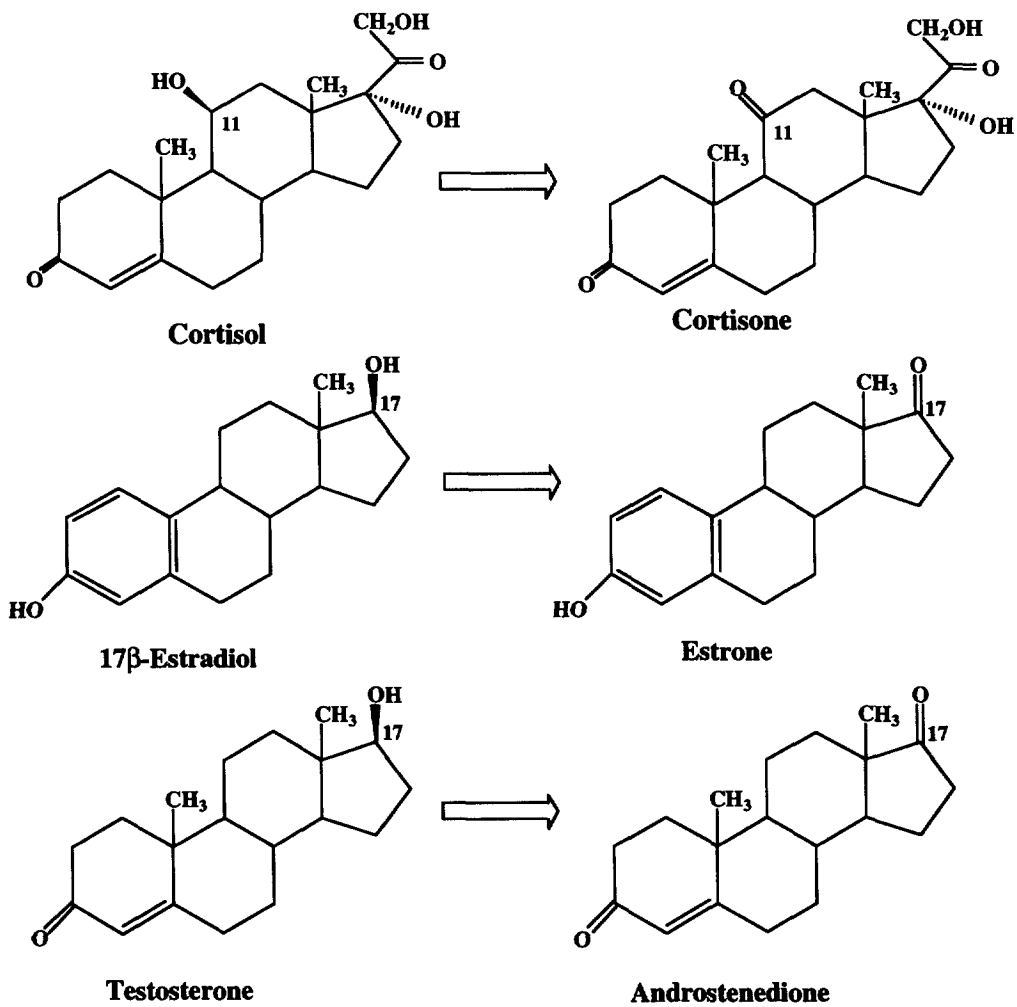

Fig. 1. Metabolism of steroids by 11β- and 17β-hydroxysteroid dehydrogenases-type 2. 11β-hydroxysteroid dehydrogenase-2 catalyzes the oxidation of cortisol to cortisone, an inactive steroid. 17β-hydroxysteroid dehydrogenase-type 2 catalyzes the oxidation of estradiol to estrone as well as the oxidation of testosterone to androstenedione.

by 17β-HSD-6 [19]. Thus, the close sequence similarity correlates with biochemical similarities.

The homology of 11β-HSD-2 and 17β-HSD-2 with all-*trans*-retinol dehydrogenase is interesting because the latter enzyme converts retinol to retinaldehyde as part of the pathway for the synthesis of retinoic acid [20, 21], a regulator of development and growth in vertebrates [4, 6]. Thus, three dehydrogenases that function as oxidases to regulate the active concentrations of retinoids and steroids are descended from a common ancestor, as are retinoid and steroid receptors [4, 6, 22, 23].

The importance of 11β-HSD-2 and 17β-HSD-2 and all-*trans*-retinol dehydrogenase in human endocrine function prompted us to investigate the evolution of 11β-HSD-2 and 17β-HSD-2 and all-*trans*-retinol dehydrogenase and their relationship to β-OH-BDH. In particular, which activity came first? Was their closest common ancestor a hydroxysteroid dehydrogenase or was it a retinoid dehydrogenase, or did it have a metabolic function? To answer this question, the Gapped–BLAST program [24] was used to search Genpept with each of the six dehydrogenases. Four open reading frames (ORFs) in *Caenorhabditis elegans* were identified as ancestral to the retinol dehydrogenases, 11β-HSD-2, 17β-HSD-2 and β-OH-BDH. A motif analysis [25–28] of the four *C. elegans* ORFs and a database search with these motifs shows that they are closest to all-*trans*-retinol dehydrogenase. The *C. elegans* proteins are about 37% to 43% identical to retinol dehydrogenase, which is sufficient to suggest that one or more of the *C. elegans* homologs metabolizes a retinoid. Retinoid receptors, but neither glucocorticoid nor sex steroid receptors have been identified in *C. elegans* [22, 23]. We propose that the 11β-HSD-2 and 17β-HSD-2 mechanism for regulating androgen, estrogen, and glucocorticoid action is descended from that for regulating retinoid action.

In the course of the analyses reported here, we found that an *E. coli* protein is phylogenetically close to animal retinol dehydrogenases. Our analyses indicate that the *E. coli* protein may be an example of horizontal transfer from a eukaryotic ancestor.

METHODS

Database searching

BLAST [29] and Gapped–BLAST searches [24] were done for each dehydrogenase on the Internet server of the National Center for Biotechnology Information. Both methods yielded a similar output (data not shown). We show the Gapped–BLAST output because this algorithm is more sensitive for finding proteins that are distantly related to the query protein [24].

Motif analysis

We used the MEME (**M**ultiple **E**xpectation maximum for **M**otif **E**licitation) algorithm to determine 9 motifs for four *C. elegans* proteins that were identified as homologous to the dehydrogenase dataset as described below. MEME has been described in detail elsewhere [25–28]. Briefly, MEME is an artificial intelligence-based motif analysis tool that takes a group of input sequences and identifies conserved regions (i.e. motifs) that are characteristic of the dataset in an automated manner without human bias. These motifs are represented as a position-dependent probability matrix or log-odds matrix, which is similar to that from a profile analysis [30]: each column of the matrix gives the probabilities of each residue in that position based on the input dataset. The log-odds matrix for the motifs generated by MEME can be ported into MAST (**M**otif **A**lignment and **S**earch **T**ool) and used to search databases such as Genpept and SWISSPROT. Usually databases are searched with the goal of finding distantly related homologs [26–28, 30]. To accomplish this, one uses motifs obtained from an analysis of a divergent collection of protein homologs, preferably with sequence identity of between 20% and 30% with each other. However, MEME and MAST can also be used in a restrictive mode, in which the sequences have between 35% and 60% sequence identity with each other. In this case, MEME will generate a log-odds matrix that is a fingerprint of the closely related protein sequences, which when used in a MAST analysis yields a output that is selective for the closest homologs in the database.

Phylogenetic analysis

The Feng–Doolittle algorithm [31] was used to construct the phylogenetic tree. In this method, first the proteins are progressively aligned using the Dayhoff PAM-250 scoring matrix to assess the pairwise similarity of each protein with the others, and the scores are assembled into a distance matrix. Then the method of Fitch and Margoliash [32] is used to obtain the best branching order for the sequences. Branch lengths are calculated by a linear regression analysis of the best fit of the pairwise distances and the branching order. The lengths of the branches are proportional to the relative distance between the sequences.

RESULTS

Gapped blast analyses

The results of Gapped–BLAST analyses [24] of six dehydrogenases are shown in Fig. 2. For clarity, scores for the same gene in different species have been deleted. For example, only human 11β-HSD-2 is shown; the scores for the mouse, rat, and rabbit

Query= DHI2_HUMAN CORTICOSTEROID 11-BETA-HYDROXYSTEROID DEHYDROGENASE TYPE 2

Sequences producing significant alignments:	Score (bits)	E Value
sp|P80365|DHI2_HUMAN CORTICOSTEROID 11-BETA-DEHYDROGENASE, ISOZ...	826	0.0
sp|P37059|DHB2_HUMAN ESTRADIOL 17 BETA-DEHYDROGENASE 2 (17-BETA...	243	1e-63
sp|P50169|ROH1_RAT ALL-*TRANS*-RETINOL DEHYDROGENASE TYPE I.....	162	2e-39
sp|Q02338|BDH_HUMAN D-BETA-HYDROXYBUTYRATE DEHYDROGENASE PRECUR...	159	3e-38
sp|Q92781|RDH1_HUMAN 11-*CIS* RETINOL DEHYDROGENASE (11-*CIS* RDH) ...	157	1e-37
gi|1916935 (U89717) 9-*cis*-retinol specific dehydrogenase [Homo ...	146	2e-34
gi|2661211 (U89281) 17-BETA hydroxysteroid dehydrogenase-TYPE6...	145	4e-34
gi|2384782 (AF022968) strong similarity to the insect-type alco...	138	4e-32
gnl|PID|e248970 (Z74032) F35B12.2 [*Caenorhabditis elegans*]	125	5e-28
gi|2088664 (AF003130) strong similarity to insect-type alcohol ...	118	7e-26
gi|746445 (U23455) similar to D-beta-hydroxybutyrate dehydroge...	116	3e-25
sp|P77388|YBBO_ECOLI HYPOTHETICAL OXIDOREDUCTASE IN USHA-TESA I..	95	6e-19
sp|Q09851|YAEB_SCHPO HYPOTHETICAL OXIDOREDUCTASE C23D3.11 IN CH..	81	9e-15
sp|P40471|YIM4_YEAST HYPOTHETICAL OXIDOREDUCTASE IN KGD1-SIM1 I..	71	1e-11
sp|Q05016|YM71_YEAST HYPOTHETICAL OXIDOREDUCTASE IN MRPL44-MTF1...	66	5e-10

Query= DHB2_HUMAN ESTRADIOL 17-BETA-HYDROXYSTEROID DEHYDROGENASE TYPE 2

Sequences producing significant alignments:	Score (bits)	E Value
sp|P37059|DHB2_HUMAN ESTRADIOL 17 BETA-DEHYDROGENASE 2 (17-BETA...	799	0.0
sp|P80365|DHI2_HUMAN CORTICOSTEROID 11-BETA-DEHYDROGENASE, ISOZ...	243	1e-63
sp|Q02338|BDH_HUMAN D-BETA-HYDROXYBUTYRATE DEHYDROGENASE PRECUR...	164	9e-40
sp|P50169|ROH1_RAT ALL-*TRANS*-RETINOL DEHYDROGENASE TYPE I.....	161	6e-39
sp|Q92781|RDH1_HUMAN 11-*CIS* RETINOL DEHYDROGENASE (11-*CIS* RDH) ...	151	7e-36
gi|2661211 (U89281) 17-BETA hydroxysteroid dehydrogenase-TYPE 6 ..	145	5e-34
gi|1916935 (U89717) 9-*cis*-retinol specific dehydrogenase [Homo ...	139	3e-32
gi|2088664 (AF003130) strong similarity to insect-type alcohol ...	138	7e-32
gi|2384782 (AF022968) strong similarity to the insect-type alco...	125	5e-28
gi|746445 (U23455) similar to D-beta-hydroxybutyrate dehydroge...	124	9e-28
gnl|PID|e248970 (Z74032) F35B12.2 [*Caenorhabditis elegans*]	107	1e-22
sp|Q09851|YAEB_SCHPO HYPOTHETICAL OXIDOREDUCTASE C23D3.11 IN CH...	89	5e-17
sp|P77388|YBBO_ECOLI HYPOTHETICAL OXIDOREDUCTASE IN USHA-TESA I...	80	2e-14
sp|P40471|YIM4_YEAST HYPOTHETICAL OXIDOREDUCTASE IN KGD1-SIM1 I...	75	9e-13
sp|Q05016|YM71_YEAST HYPOTHETICAL OXIDOREDUCTASE IN MRPL44-MTF1...	64	2e-09

Query= gi|2661211 17-BETA HYDROXYSTEROID DEHYDROGENASE-TYPE 6

Sequences producing significant alignments:	Score (bits)	E Value
gi|2661211 (U89281) 17-BETA hydroxysteroid dehydrogenase-TYPE 6...	661	0.0
sp|P50169|ROH1_RAT ALL-*TRANS*-RETINOL DEHYDROGENASE TYPE I.....	415	e-115
sp|Q92781|RDH1_HUMAN 11-*CIS* RETINOL DEHYDROGENASE (11-*CIS* RDH) ...	291	3e-78
gi|1916935 (U89717) 9-*cis*-retinol specific dehydrogenase [Homo ...	283	1e-75
gi|2088664 (AF003130) strong similarity to insect-type alcohol ...	194	4e-49
gi|2384782 (AF022968) strong similarity to the insect-type alco...	192	2e-48
gnl|PID|e248970 (Z74032) F35B12.2 [*Caenorhabditis elegans*]	189	1e-47
sp|Q02338|BDH_HUMAN D-BETA-HYDROXYBUTYRATE DEHYDROGENASE PRECUR...	171	4e-42
gi|746445 (U23455) similar to D-beta-hydroxybutyrate dehydroge...	169	1e-41
sp|P80365|DHI2_HUMAN CORTICOSTEROID 11-BETA-DEHYDROGENASE, ISOZ...	145	3e-34
sp|P37059|DHB2_HUMAN ESTRADIOL 17 BETA-DEHYDROGENASE 2 (17-BETA...	145	4e-34
sp|P77388|YBBO_ECOLI HYPOTHETICAL OXIDOREDUCTASE IN USHA-TESA I...	90	2e-17
sp|Q09851|YAEB_SCHPO HYPOTHETICAL OXIDOREDUCTASE C23D3.11 IN CH...	73	2e-12
sp|Q05016|YM71_YEAST HYPOTHETICAL OXIDOREDUCTASE IN MRPL44-MTF1...	65	5e-10

Fig. 2 *continued on opposite page.*

Query= ROH1_RAT ALL-*TRANS*-RETINOL DEHYDROGENASE TYPE I (RODH I)

Sequences producing significant alignments:	Score (bits)	E Value
sp|P50169|ROH1_RAT ALL-*TRANS*-RETINOL DEHYDROGENASE TYPE I.....	647	0.0
gi|2661211 (U89281) 17-BETA hydroxysteroid dehydrogenase-TYPE 6...	415	e-115
sp|Q92781|RDH1_HUMAN 11-*CIS* RETINOL DEHYDROGENASE (11-*CIS* RDH) ...	340	7e-93
gi|1916935 (U89717) 9-*cis*-retinol specific dehydrogenase [Homo ...	329	1e-89
gi|2384782 (AF022968) strong similarity to the insect-type alco...	227	7e-59
gi|2088664 (AF003130) strong similarity to insect-type alcohol ...	216	1e-55
gnl|PID|e248970 (Z74032) F35B12.2 [*Caenorhabditis elegans*]	215	2e-55
gi|746445 (U23455) similar to D-beta-hydroxybutyrate dehydroge...	203	1e-51
sp|Q02338|BDH_HUMAN D-BETA-HYDROXYBUTYRATE DEHYDROGENASE PRECUR...	202	2e-51
sp|P80365|DHI2_HUMAN CORTICOSTEROID 11-BETA-DEHYDROGENASE, ISOZ...	162	2e-39
sp|P37059|DHB2_HUMAN ESTRADIOL 17 BETA-DEHYDROGENASE 2 (17-BETA...	161	5e-39
sp|P77388|YBBO_ECOLI HYPOTHETICAL OXIDOREDUCTASE IN USHA-TESA I...	111	7e-24
sp|Q09851|YAEB_SCHPO HYPOTHETICAL OXIDOREDUCTASE C23D3.11 IN CH...	92	5e-18
sp|Q05016|YM71_YEAST HYPOTHETICAL OXIDOREDUCTASE IN MRPL44-MTF1...	82	5e-15

Query= RDH1_HUMAN 11-*CIS* RETINOL DEHYDROGENASE (11-*CIS* RDH)

Sequences producing significant alignments:	Score (bits)	E Value
sp|Q92781|RDH1_HUMAN 11-*CIS* RETINOL DEHYDROGENASE (11-*CIS* RDH) ...	612	e-175
gi|1916935 (U89717) 9-*cis*-retinol specific dehydrogenase [Homo ...	594	e-169
sp|P50169|ROH1_RAT ALL-*TRANS*-RETINOL DEHYDROGENASE TYPE I.....	327	6e-89
gi|2661211 (U89281) 17-BETA hydroxysteroid dehydrogenase-TYPE 6...	285	3e-76
gi|2088664 (AF003130) strong similarity to insect-type alcohol ...	212	3e-54
gi|2384782 (AF022968) strong similarity to the insect-type alco...	199	2e-50
gnl|PID|e248970 (Z74032) F35B12.2 [*Caenorhabditis elegans*]	185	3e-46
gi|746445 (U23455) similar to D-beta-hydroxybutyrate dehydroge...	171	3e-42
sp|Q02338|BDH_HUMAN D-BETA-HYDROXYBUTYRATE DEHYDROGENASE PRECUR...	167	7e-41
sp|P80365|DHI2_HUMAN CORTICOSTEROID 11-BETA-DEHYDROGENASE, ISOZ...	152	2e-36
sp|P37059|DHB2_HUMAN ESTRADIOL 17 BETA-DEHYDROGENASE 2 (17-BETA...	148	3e-35
sp|P77388|YBBO_ECOLI HYPOTHETICAL OXIDOREDUCTASE IN USHA-TESA I...	127	6e-29
sp|Q09851|YAEB_SCHPO HYPOTHETICAL OXIDOREDUCTASE C23D3.11 IN CH...	92	5e-18
sp|Q05016|YM71_YEAST HYPOTHETICAL OXIDOREDUCTASE IN MRPL44-MTF1...	85	4e-16

Query= BDH_HUMAN D-BETA-HYDROXYBUTYRATE DEHYDROGENASE PRECURSOR (BDH)

Sequences producing significant alignments:	Score (bits)	E Value
sp|Q02338|BDH_HUMAN D-BETA-HYDROXYBUTYRATE DEHYDROGENASE PRECUR...	710	0.0
sp|P50169|ROH1_RAT ALL-*TRANS*-RETINOL DEHYDROGENASE TYPE I.....	202	3e-51
gnl|PID|e248970 (Z74032) F35B12.2 [*Caenorhabditis elegans*]	173	1e-42
gi|2661211 (U89281) 17-BETA hydroxysteroid dehydrogenase-TYPE 6...	171	5e-42
sp|Q92781|RDH1_HUMAN 11-*CIS* RETINOL DEHYDROGENASE (11-*CIS* RDH) ...	167	7e-41
sp|P37059|DHB2_HUMAN ESTRADIOL 17 BETA-DEHYDROGENASE 2 (17-BETA...	164	8e-40
gi|2088664 (AF003130) strong similarity to insect-type alcohol ...	161	4e-39
sp|P80365|DHI2_HUMAN CORTICOSTEROID 11-BETA-DEHYDROGENASE, ISOZ...	159	3e-38
gi|1916935 (U89717) 9-*cis*-retinol specific dehydrogenase [Homo ...	158	5e-38
gi|2384782 (AF022968) strong similarity to the insect-type alco...	148	4e-35
gi|746445 (U23455) similar to D-beta-hydroxybutyrate dehydroge...	142	2e-33
sp|P77388|YBBO_ECOLI HYPOTHETICAL OXIDOREDUCTASE IN USHA-TESA I...	112	3e-24
sp|Q09851|YAEB_SCHPO HYPOTHETICAL OXIDOREDUCTASE C23D3.11 IN CH...	89	3e-17
sp|Q05016|YM71_YEAST HYPOTHETICAL OXIDOREDUCTASE IN MRPL44-MTF1...	81	1e-14

Fig. 2. Gapped-BLAST analysis of 11β-HSD-2 and 17β-HSD-2, 17β-HSD-6, all-*trans*- and 11-*cis*-retinol dehydrogenases and β-OH-BDH. The output of a Gapped-BLAST search of Genpept with each enzyme is shown. Scores for sequences from the same gene in a different species are deleted. Thus, only the score for human 11β-HSD-2 is shown, which is similar to that of mouse, rat or sheep 11β-HSD-2.

genes, which are similar to that of the human gene, are deleted.

Gapped-BLAST analysis of human 11β-HSD-2 shows it is closest to the 17β-HSD-2. Then there is a sharp decline in the scores with rat all-*trans*-retinol dehydrogenase, human β-OH-BDH, human 11-*cis*-retinol dehydrogenase, human 9-*cis* retinol dehydrogenase and human 17β-HSD-6 clustering close together. The next group contains *C. elegans* AF022968, F35B12.2, AF00310 and U23455. Also

note that *E. coli* YBBO has an *E* value of 6×10^{-19}, which puts it closer to 11β-HSD-2 than either *Schizosaccharomyces pombe* YAEB or *Saccharomyces cerevisiae* YIM4 and YM71. As will be seen below, the Gapped–BLAST analyses indicate that YBBO is unusually close to retinol dehydrogenases.

Gapped–BLAST analysis of human 17β-HSD-2 shows that it is closest to 11β-HSD-2. A little more distant are β-OH-BDH, retinol dehydrogenases and 17β-HSD-6. The four *C. elegans* proteins are most distantly related to 17β-HSD-2.

Gapped–BLAST analysis of human 17β-HSD-6 shows it is closest to retinol dehydrogenases, as reported previously [19]. Next closest are the four *C. elegans* proteins and β-OH-BDH. Most distant are 17β-HSD-2 and 11β-HSD-2.

Gapped–BLAST analyses of the two retinol dehydrogenases show that they are closer to the four *C. elegans* proteins than to either 11β-HSD-2 or 17β-HSD-2. Moreover, the score of human 11-*cis*-retinol dehydrogenase with *C. elegans* AF003130 is 3×10^{-54}. In contrast, 11-*cis*-retinol dehydrogenase has scores of 2×10^{-36} and 3×10^{-35}, respectively, with 11β-HSD-2 and 17β-HSD-2. Not much more distant is *E. coli* YBBO with a score of 6×10^{-29}. The yeast proteins are substantially more distant.

Gapped–BLAST analysis of β-OH-BDH shows it is closest to the retinol dehydrogenases, *C. elegans* F35B12.2, 17β-HSD-6, 17β-HSD-2 and AF003130. β-OH-BDH is closer to 11β-HSD-2 than to *C. elegans* U23455.

Gapped–BLAST analyses of the four *C. elegans* proteins are consistent with the results of the analyses of retinol dehydrogenases (data not shown). The *C. elegans* proteins are closest to each other; their next closest proteins are the retinol dehydrogenases, 17β-HSD-6 and β-OH-BDH.

Motif analysis

To investigate the relationship of the four *C. elegans* proteins to other dehydrogenases, we used MEME to determine 9 motifs that characterize the four *C. elegans* proteins. The log-odds matrix for these motifs was used by MAST to search Genpept. The output shown in Fig. 3 reveals that four *C. elegans* proteins cluster together, with the next closest proteins being the retinol dehydrogenases and 17β-HSD-6, which is 65% identical to retinol dehydrogenase [19]. β-OH-BDH, 11β-HSD and 17β-HSD are clearly more distant. Surprisingly, *E. coli* YBBO has a score of 4.7×10^{-14}, which places this protein closer to the *C. elegans* proteins than either mouse or human 17β-HSD-2. *S. cerevisiae* YM71 has a score of 3.2×10^{-8}. Thus, *E. coli* YBBO is closer to the *C. elegans* proteins than to any other unicellular eukaryote in the database. Moreover, a Gapped–BLAST analysis of the yeast protein indicates that it is closest to bacterial proteins and not a close ancestor to any mammalian dehydrogenase (data not shown). This suggests an unusual evolution of *E. coli* YBBO.

Relationship of E. coli YBBO to retinol dehydrogenases

To examine further the relationship of *E. coli* YBBO, we did a Gapped–BLAST search of Genpept for homologs of YBBO. As seen in Fig. 4, YBBO has *E* values of 10^{-29} to 10^{-28} to a protein in *Helicobacter pylori* and to several retinol dehydrogenases. The Gapped–BLAST search did not find any yeast protein that is closer to YBBO than are the retinol dehydrogenases.

SEQUENCE NAME	DESCRIPTION	E-VALUE	LENGTH
gnl|PID|e248970	(Z74032) F35B12.2 [Caenorhabditis e...	2e-96	374
gi|2384782	(AF022968) strong similarity to the...	1.3e-93	388
gi|746445	(U23455) similar to D-beta-hydroxy...	2.1e-90	305
gi|2088664	(AF003130) strong similarity to ins...	1.e-89	343
sp|P50169|ROH1_RAT	RETINOL DEHYDROGENASE TYPE I (RODH ...	2e-49	317
sp|Q92781|RDH1_HUMAN	11-CIS RETINOL DEHYDROGENASE (11-CI...	4.8e-43	318
gi|1916935	(U89717) 9-cis-retinol specific deh...	1.5e-41	318
gi|2661211	(U89281) 17-BETA HSD-TYPE 6 ...	2e-41	317
sp|Q02338|BDH_HUMAN	D-BETA-HYDROXYBUTYRATE DEHYDROGENAS...	2.8e-30	343
sp|P80365|DHI2_HUMAN	CORTICOSTEROID 11-BETA-DEHYDROGENAS...	3.7e-21	405
sp|P77388|YBBO_ECOLI	HYPOTHETICAL OXIDOREDUCTASE IN USHA...	4.4e-14	269
sp|P51658|DHB2_MOUSE	ESTRADIOL 17 BETA-DEHYDROGENASE 2 ...	5.5e-13	381
gnl|PID|e1173565	(Z93941) YuxA [Bacillus subtilis]gi...	7.1e-11	280
sp|O05730|VDLC_HELPY	PROBABLE SHORT-CHAIN TYPE DEHYDROGE...	1.6e-10	284
gnl|PID|e1228151	(AJ000671) clavulanate-9-aldehyde r...	4.2e-10	247
sp|P37059|DHB2_HUMAN	ESTRADIOL 17 BETA-DEHYDROGENASE 2 ...	6.7e-10	387
sp|Q05016|YM71_YEAST	HYPOTHETICAL OXIDOREDUCTASE IN MRPL...	3.6e-08	267

Fig. 3. MAST search of Genpept with MEME motifs of four *C. elegans* dehydrogenase homologs. The four *C. elegans* proteins cluster together. The next closest sequences are retinol dehydrogenases and 17β-HSD-6. β-OH-BDH and 11β-HSD-2 and 17β-HSD-2 are clearly more distant. *E. coli* YBBO has a slightly higher score than 17β-HSD-2.

```
                                                                    Score     E
Sequences producing significant alignments:                         (bits)  Value

sp|P77388|YBBO_ECOLI  HYPOTHETICAL OXIDOREDUCTASE IN USHA-TESA I...540  e-153
sp|O05730|VDLC_HELPY  PROBABLE SHORT-CHAIN TYPE DEHYDROGENASE/RE...129  1e-29
sp|Q92781|RDH1_HUMAN  11-CIS RETINOL DEHYDROGENASE (11-CIS RDH) ...127  5e-29
gi|1916935  (U89717) 9-cis-retinol specific dehydrogenase [Homo ...124  4e-28
sp|Q02338|BDH_HUMAN  D-BETA-HYDROXYBUTYRATE DEHYDROGENASE PRECUR...112  2e-24
gi|2661213  (U89280) 17 beta hydroxysteroid dehydrogenase type 6...111  3e-24
pir||I40869  ORF4 - Clostridium perfringens (fragment) >gi|85381...111  5e-24
sp|P50169|ROH1_RAT  RETINOL DEHYDROGENASE TYPE I (RODH I) >gi|10...111  5e-24
sp|P54554|YQJQ_BACSU  HYPOTHETICAL OXIDOREDUCTASE IN GLNQ-ANSR I...108  3e-23
gi|2314026  (AE000598) conserved hypothetical protein [Helicobac...104  5e-22
gi|2338748  (AF016509) oxidoreductase [Homo sapiens]                 103  1e-21
gnl|PID|e1173565  (Z93941) YuxA [Bacillus subtilis] >gi|2635794|...103  1e-21
gnl|PID|d1020344  (AB002410) 17-beta-hydroxysteroid dehydrogenas...102  2e-21
gnl|PID|e316922  (Z95556) unknown [Mycobacterium tuberculosis]       97  9e-20
sp|P80365|DHI2_HUMAN  CORTICOSTEROID 11-BETA-DEHYDROGENASE, ISOZ...  95  3e-19
```

Fig. 4. Gapped-BLAST analysis of *E. coli* YBBO.

Next we used MEME to determine nine motifs in rat all-trans-retinol DH, human 11-*cis*-retinol dehydrogenase and 17β-HSD-6. As seen in Table 1, these proteins have between 47% and 62% sequence identity. Thus, the MEME motifs will be very specific for these retinol dehydrogenases. The output of a search of Genpept with these nine motifs using MAST is shown in Fig. 5. As expected the retinol dehydrogenase cluster together with E values of 10^{-70} or lower. Then there is a break in the scores, with the *C. elegans* homologs and β-OH-BDH clustering together with E values of 10^{-18}–10^{-27}. The next set includes human 11β-HSD-2, *E. coli* YBBO and mouse 17β-HSD-2 with E values from 10^{-8} to 10^{-11}. *S. cerevisiae* YM71 has an E value of 3.8×10^{-2}; *H. pylori* VDLC has an E value of 2×10^{-1}. Thus, the MAST analysis confirms the unusual closeness of *E. coli* YBBO to retinol dehydrogenases.

Phylogenetic analysis

The evolutionary relationship of the *C. elegans* proteins and their dehydrogenase homologs and *E. coli* YBBO is shown in Fig. 6. Table 1 shows the % identity between the sequences used to construct the phylogenetic tree. The tree clearly shows that all-*trans*- and 11-*cis*-retinol dehydrogenases and 17β-HSD-6 cluster with the four *C. elegans* proteins. Next closest is β-OH-BDH followed by 11β-HSD-2 and 17β-HSD-2. *E. coli* YBBO is most distant. Summation of the branch lengths provides a measure of the relative distance these proteins from each other. For example, *E. coli* YBBO and 17β-HSD-2 are 116.4 units and 103.9 units, respectively from all-*trans*-retinol dehydrogenase. 17β-HSD-2 is 132.3 units from YBBO.

DISCUSSION

Using Gapped–BLAST and Motif database searches and a phylogenetic analysis, we have investigated the evolutionary origins of all-*trans*- and 11-*cis*-retinol dehydrogenase, 11β-HSD-2 and 17β-HSD-2 and β-OH-BDH. Gapped–BLAST identifies four ORFs, F35B12.2, U23455, AF022968 and AF003130, from *C. elegans* as homologs of these dehydrogenases. In Genpept, F35B12.2 is identified as a retinol dehydrogenase homolog; U23455 is identified as a β-OH-BDH homolog, and AF022968 and AF003130 are identified as an insect short-chain dehydrogenase homologs.

The Gapped–BLAST, motif and phylogenetic analyses place the *C. elegans* ORFs closest to all-*trans*-retinol dehydrogenase. Biswas and Russell reported

Table 1. *Identical amino acids (%) in the sequence alignment used to construct the phylogenetic tree shown in Fig. 6*

	All-*trans* RDH	17β-HSD-6	11-*cis*-RDH	AF003130	U23455	F35B12.2	AF022968	11β-HSD-2	17β-HSD-2	β-OH-BDH
All-*trans*-RDH	100	–	–	–	–	–	–	–	–	–
17β-HSD-6	62.5	100	–	–	–	–	–	–	–	–
11-*cis*-RDH	52.6	47.4	100	–	–	–	–	–	–	–
AF003130	40.7	36.1	38.8	100	–	–	–	–	–	–
U23455	40.0	34.8	36.6	42.2	100	–	–	–	–	–
F35B12.2	42.8	37.9	37.1	43.4	41.5	100	–	–	–	–
AF022968	41.8	37.9	37.1	43.2	39.0	44.4	100	–	–	–
11β-HSD-2	33.1	30.0	32.4	28.8	26.8	31.1	31.6	100	–	–
17β-HSD-2	32.8	28.7	30.5	30.0	26.8	27.7	29.3	44.9	100	–
β-OH-BDH	40.6	37.0	34.8	34.5	33.0	37.3	34.4	33.7	34.4	100
E. coli YBBO	31.5	27.6	31.9	29.2	28.4	30.5	28.0	31.1	27.5	30.5

SEQUENCE NAME	DESCRIPTION	E-VALUE	LENGTH
gi|2661211	(U89281) 17-BETA HSD-TYPE 6	1.5e-92	317
sp|P50169|ROH1_RAT	RETINOL DEHYDROGENASE TYPE I (RODH ...	4.6e-91	317
sp|Q92781|RDH1_HUMAN	11-CIS RETINOL DEHYDROGENASE (11-CI...	1.6e-76	318
gi|1916935	(U89717) 9-cis-retinol specific deh...	2.7e-70	318
gnl|PID|e248970	(Z74032) F35B12.2 [Caenorhabditis e...	3.9e-27	374
sp|Q02338|BDH_HUMAN	D-BETA-HYDROXYBUTYRATE DEHYDROGENAS...	4e-26	343
gi|2088664	(AF003130) strong similarity to ins...	6.4e-24	343
gi|2384782	(AF022968) strong similarity to the...	8.7e-23	388
gi|746445	(U23455) similar to D-beta-hydroxy...	4.1e-18	305
sp|P80365|DHI2_HUMAN	CORTICOSTEROID 11-BETA-DEHYDROGENAS...	1.8e-11	405
sp|P77388|YBBO_ECOLI	HYPOTHETICAL OXIDOREDUCTASE IN USHA...	2.5e-09	269
gi|1200097	(X95685) 17-beta-HSD-2 Mouse ...	7.1e-08	358
gi|559964	(J05282) insect-type dehydrogenase ...	4e-05	249
sp|P14061|DHB1_HUMAN	ESTRADIOL 17 BETA-DEHYDROGENASE 1 ...	5.7e-05	328
sp|P37059|DHB2_HUMAN	ESTRADIOL 17 BETA-DEHYDROGENASE 2 ...	0.00018	387
gnl|PID|e1173565	(Z93941) YuxA [Bacillus subtilis]gi..	0.00041	280
sp|Q05016|YM71_YEAST	HYPOTHETICAL OXIDOREDUCTASE IN MRPL...	0.038	267
sp|O05730|VDLC_HELPY	PROBABLE SHORT-CHAIN TYPE DEHYDROGE...	0.2	284

Fig. 5. MAST search of Genpept with MEME motifs of rat all-*trans*-retinol dehydrogenase, human 11-*cis*-retinol dehydrogenase and human 17β-hydroxysteroid dehydrogenase-type 6. *E. coli* YBBO has an *E* value of 2.5×10^{-9}, which places it unusually close to the retinol dehydrogenases. In contrast, *S. cerevisiae* YM71 has an *E* value of 3.8×10^{-2}.

that all-*trans*-retinol dehydrogenase has 17β-HSD activity and that retinoids bind to 17β-HSD-6 [19]. This is consistent with the strong sequence similarity between retinol dehydrogenases and 17β-HSD-6 [19] and 17β-HSD-2 [16, 33]. And it is consistent with evidence that homologous proteins in different species with as low as 35% to 40% sequence identity can have similar catalytic activity [34, 35].

The activities of the four *C. elegans* ORFs are not known. However, based on the above, the four *C. elegans* proteins have sufficient sequence similarity to all-*trans*-retinol dehydrogenase to metabolize a retinoid that activates a nuclear receptor. Supporting this possibility is the existence of receptors that are homologs of retinoic acid receptor and retinoid X receptor in *C. elegans* and other invertebrates [4, 22, 23].

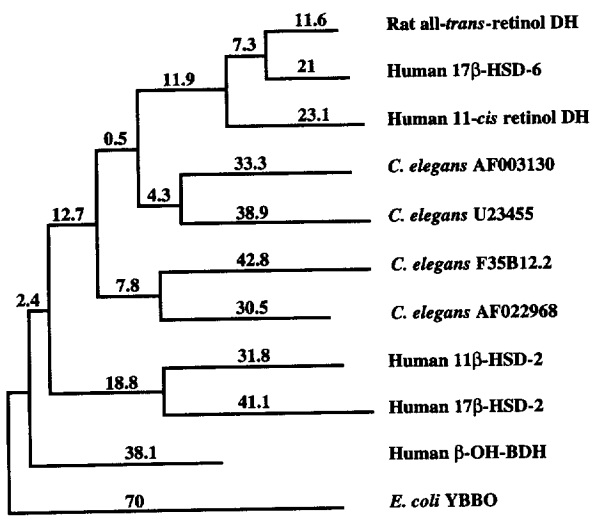

Fig. 6. Phylogeny of human 11β- and 17β-hydroxysteroid and retinol dehydrogenases and homologs in *C. elegans* and *E. coli*. The branch distances can be used to calculate the relative distances of each protein from each other. For example *E. coli* YBBO is 116.4 units (70 + 2.4 + 12.7 + 0.5 + 11.9 + 7.3 + 11.6) and human 17β-HSD-2 is 103.9 units (41.1 + 18.8 + 12.7 + 0.5 + 11.9 + 7.3 + 11.6) from rat all-*trans*-retinol dehydrogenase.

The phylogenetic analysis and database searches presented here indicate that the type-2 dehydrogenases that regulate the access of active adrenal and sex steroids to cells are descended from enzymes that metabolize retinoids. Sequence analyses of adrenal and sex steroid receptors support this hypothesis. Thus far, fish have been found to contain estrogen [36] and glucocorticoid [37] receptors, but homologs of neither receptor has been found in either *C. elegans* or other invertebrates. We have proposed that adrenal and sex steroid receptors arose in a tunicate or *Amphioxus* ancestor of vertebrates [38] and that these receptors will not be found in *C. elegans*. At this time, about 75% of the *C. elegans* genome is sequenced, with completion expected in six months to a year. Complete sequencing of the *C. elegans* genome and the eventual sequencing of the *Drosophila* genome will clarify the origins of the receptors and enzymes involved in regulation of retinoid and adrenal and sex steroid action in humans.

Some of the mammalian dehydrogenases in Fig. 6 appear to have multiple activities, as seen for 17β-HSD-2 [12] and retinol dehydrogenase [19] or are inhibited by compounds distinctly different from their canonical substrate, as seen for 17β-HSD-6 [19]. This raises the possibility that β-OH-BDH, which has about 40% sequence identity to all-*trans*-retinol dehydrogenase, may metabolize substrates other than β-OH-butyrate. It may also explain recent evidence that novel steroidal substrates inhibit 11β-HSD-2 [39].

When *E. coli* YBBO was sequenced, it was entered into Genpept as a homolog of 11-*cis*-retinol dehydrogenase, based on a Gapped–BLAST analysis. One interpretation of this similarity is that *E. coli* YBBO is a prokaryote ancestor of the retinol dehydrogenases, 11β-HSD-2 and 17β-HSD-2. However, the Gapped–BLAST analysis shown in Fig. 4 and the MAST search of Genpept with retinol dehydrogenase motifs shown in Fig. 5 clearly place *E. coli* YBBO closer to mammalian dehydrogenases than to any homolog in yeast or any other unicellular eukaryote. Thus, it may be that *E. coli* YBBO arose by horizontal transfer from a eukaryote ancestor to a prokaryote. This leaves the more ancient origins of retinol dehydrogenases unanswered, as well as the origins and function of *E. coli* YBBO.

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