Molecular Characterization of a Mouse Short Chain Dehydrogenase/Reductase Active with All-trans-retinol in Intact Cells, mRDH1*

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Metabolic activation of retinol (vitamin A) via sequential actions of retinol and retinol dehydrogenases produces the active metabolite all-trans-retinoic acid. This work reports cDNA cloning, enzymatic characterization, function in a reconstituted path of all-trans-retinoic acid biosynthesis in cell culture, and mRNA expression patterns in adult tissues and embryos of a mouse retinol dehydrogenase, RDH1. RDH1 represents a new member of the short chain dehydrogenase/reductase superfamily that differs from other mouse RDH in relative activity with all-trans and cis-retinols. RDH1 has a multifunctional catalytic nature, as do other short chain dehydrogenase/reductases. In addition to retinol dehydrogenase activity, RDH1 has strong 3α-hydroxy and weak 17β-hydroxy steroid dehydrogenase activities. RDH1 has widespread and intense mRNA expression in tissues of embryonic and adult mice. The mouse embryo expresses RDH1 as early as 7.0 days post-coitus, and expression is especially intense within the neural tube, gut, and neural crest at embryonic day 10.5. Cells cotransfected with RDH1 and any one of three retinal dehydrogenase isozymes synthesize all-trans-retinoic acid from retinol, demonstrating that RDH1 contributes to a path of all-trans-retinoic acid biosynthesis intact cells. These characteristics are consistent with RDH1 functioning in a path of all-trans-retinoic acid biosynthesis starting early during embryogenesis.

Naturally occurring retinoids (vitamin A and its metabolites) function beyond their well known contributions to vision, conception, growth, and epithelial differentiation. Vertebrates require retinoids for the development of numerous embryonic structures (e.g. limbs, nervous system, heart, kidney), the immune response, and control of intermediary metabolism (1–4). Retinol metabolites serve as endocrine factors that bind to and activate the RARα, -β, and -γ, and the RXRα, -β, and -γ members of the nuclear hormone receptor superfamily (5, 6). Mechanisms for the pleiotropic actions of retinoids are provided by the number of retinoid receptors, their many cell-specific isoforms (generated by differential promoter use and alternative splicing), and heterodimerization of RXR with several other nuclear receptors (7). Receptor expression patterns alone, however, do not explain fully the complex temporal and spatial effects of retinoids during embryogenesis and during postnatal development and growth (8).

Metabolism activates vitamin A (retinol) by producing atRA,1 an endogenous RAR ligand generated both centrally and locally (9, 10). Two sequential reactions produce atRA from all-trans-retinol; they are reversible and rate-limiting dehydrogenation into all-trans-retinal catalyzed by RDH and irreversible and perhaps rate-determining dehydrogenation of all-trans-retinal catalyzed by RALDH. Members of two classes of alcohol dehydrogenases have been proposed to serve as RDH (11, 12). The medium chain dehydrogenases, ADH classes I and IV, convert retinol into retinal in vitro. These enzymes belong to a family that largely detoxifies xenobiotics and have kinetic characteristics more consistent with xenobiotic clearance than for producing endocrine factors (13). ADH1 expression first appears at e10.5 and does not correlate well with sites of atRA synthesis in the mouse embryo, prompting the conclusion that its involvement appears unlikely in embryonic atRA biosynthesis (14). ADHV shows more widespread expression than ADH1, but it is not expressed in diverse areas of atRA biosynthesis and use in the adult (15). ADHV is expressed episodically during mouse embryogenesis; it is notably absent at various loci and times of atRA need. These considerations indicate that ADH1 and ADHV do not function universally to generate endocrine levels of atRA.

Pursuit of universal RDH candidates has identified several previously unknown members of the SDR superfamily (11). This phylogenetically diverse enzyme family consists of numerous mammalian members that regulate the concentrations of estrogens, androgens, glucocorticoids, and prostaglandins (16). Three SDR isozymes, RoDH1, -2, and -3, have been identified as RDH candidates in the rat. Each shares similar substrate specificities and has greater activity for all-trans-retinol than for cis-retinols (17). Each has a distinct pattern of mRNA expression. The precise functions of each have not been established in part because mouse orthologs have not been identified. As for retinoid-specific receptors and binding proteins, an RDH physiologically significant in atRA biosynthesis should be

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EBI Data Bank with accession number(s) AF028928.

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1 The abbreviations used are: atRA, all-trans-RA; 3-adiol, 5α-androstan-3α,17β-diol; DHT, dihydrotestosterone; e-, embryo days post-coitus; HPLC, high performance liquid chromatography; HSD, hydroxy- steroid dehydrogenase; RALDH, retinal dehydrogenase; RACE, rapid amplification of cDNA ends; RA, retinoic acid; RDH, retinol dehydrogenase(s); PCR, polymerase chain reaction; SDR, short chain dehydrogenase/reductase(s); bp, base pair(s); kb, kilobase(s); CHO, Chinese hamster ovary; UTR, untranslated region; ADH, alcohol dehydrogenase; CRAD, cis-retinol/androgen dehydrogenase.
Mouse Retinol Dehydrogenase Type 1 (RDH1)

**Materials and Methods**

cDNA Encoding Mouse RDH1—Degenerate primers for reverse transcription-PCR were designed from conserved amino acid sequences of rod RDH and mouse CRAD members of the SDR superfamily. The sense primer F1 (5′-GGCAGTGTTTGCAGCTTGGG-3′) and antisense primer R1 (5′-AGTCCATCGATGCTAGAC-3′) corresponded to amino acids 105–114, G(L/F)WG(L/F)VN-(A/G)TCCAT(A/G)CA, from CMEHALT(A/S); R7 (5′-ATCACTATCG-3′), to SDR amino acids FITGCDSGFG; F11 (5′-ATGAACTCCTGAGGAGGC-GAGCTTCA-3′) using mouse e17 mRNA (Sigma) as template. Reverse transcription was done with the Molec 

**Expression of RDH1—CHO-K1 cells (ATCC) were cultured at 37 °C in Ham’s F-12 medium supplemented with 10% fetal calf serum. Cells were transfected with 8 µg/100-mm plate of pcDNA3/RDH1 using Li 

**Enzyme Assays—**Kinetic data were generated from retinoids with 5 µg of CHO cell supernatant protein for 5 min and with steroids using 1 µg of protein and 3 min. These values were in the linear ranges of protein and time (initial velocity conditions) and produced easily quantifiable amounts of products. Kinetic data were fit with the nonlinear regression program GraphFit 4. RDH assays were done in triplicate at 37 °C in 0.25 ml of 50 mM Hepes, 150 mM KCl, 1 mM EDTA, pH 8. Reactions were quenched for incubation for 30 min at room
temperature with 0.3 ml of 0.1 M o-ethylhydroxylamine in 0.1 M Hepes. The aqueous phase was extracted twice with 2.5 ml of hexane. The organic phase was evaporated with nitrogen, and residues were dissolved in 0.1 ml of hexane. Retinal oximes were quantified by HPLC as described (18). Steroid dehydrogenase assays were done with [3H] steroids (40–101 Ci/mmol, 20,000 dpm/reaction) under the same conditions. Reactions were quenched with dichloromethane (3 ml), and steroids were extracted as described (18). Organic phases were evaporated with nitrogen, and the residues were dissolved in 30 μl of ethanol and applied to 1) aluminum oxide thin-layer chromatography plates developed with chloroform/ethyl acetate (3/1, v/v) to analyze conversion of androsterone into androstanedione, 3α-adiol into dihydrotestosterone, dihydrotestosterone into androstanedione, and testosterone into androstanedione; 2) silica plates developed with the same mobile phase to analyze conversion of 21-estradiol into estrone; 3) silica plates developed with chloroform/ethanol (96/4; v/v) to analyze conversion of corticosterone into 11-dehydrocorticosterone. [3H] Steroids were detected by autoradiography. Radioactive zones were excised and counted with a liquid scintillation counter.

Northern Blots—Mouse liver and kidney total RNA were extracted with Trizol reagent (Life Technologies, Inc.), and 125 μg from each were run on a formaldehyde gel, transferred to a nylon membrane, and probed with the 3'-UTR probe described below. Prehybridization was done in 10 ml of prehybridization solution (50% formamide, 5× saline/ sodium phosphate/EDTA, 10× Denhardt's solution, 2% SDS, and 100 μg/mL of denatured salmon sperm DNA) at 42°C overnight. Hybridization was done with labeled cDNA probe at 42°C for 24 h. The blot was washed three times in 1× SSC (1× SSC = 0.15 M NaCl and 0.015 M sodium citrate) with 0.1% SDS at room temperature for 30 min followed by two washes in 0.1× SSC with 0.1% SDS for 30 min at 65°C and was exposed to x-ray film. After stripping the mRDH1 probe, the blot was reprobed with a β-actin probe (CLONTECH).

Mouse Retinol Dehydrogenase Type 1 (RDH1)
RESULTS AND DISCUSSION

**cDNA Cloning**—To obtain mouse RDH1, degenerate reverse transcription-PCR was done with mRNA from e17 mice. Primers were designed from conserved regions of RoDH/CRAD members of the SDR superfamily. A 550-bp cDNA (fragment A) amplified by primers F1 and R1 had 89 and 85% nucleotide identity, respectively, with mouse CRAD1 and rat RoDH2 (Fig. 1). The sequence of this partial cDNA was used to design primers that encoded 317 amino acid residues

**TABLE I**

| Enzyme       | Nucleotide identity | Amino acid homology | GenBank accession No. |
|--------------|---------------------|---------------------|-----------------------|
| mRDH1        | 100                 | 100                 | AY039285              |
| mCRAD1       | 93                  | 90                  | AF030513              |
| mCRAD3       | 92                  | 89                  | AF372638              |
| rRoDH2       | 89                  | 84                  | U35500                |
| rRoDH3       | 87                  | 82                  | U35501                |
| mCRAD2       | 87                  | 81                  | AB032055              |
| rRoDH1       | 86                  | 81                  | U18762                |
| hRDH-E       | 79                  | 74                  | AF086735              |
| m17β-HSD9    | 72                  | 68                  | AF103797              |
| r17β-HSD6    | 71                  | 66                  | U39203                |
| hRoDH        | 70                  | 62                  | U39203                |
| b11-cis-RoDH | 61                  | 53                  | X92825                |
| mRDH4        | 60                  | 52                  | AF013288              |
| hRDH5        | 60                  | 53                  | U97177/U34559         |
| hPR-RDH      | 46                  | 18                  | AF239845              |
| mretSDR1     | 43                  | 20                  | X92821                |
| m11β-HSD1    | 43                  | 18                  | X83202                |
| m3β-HSD      | 42                  | 13                  | M58567                |

**to the manufacturer’s protocol (CLONTECH). Briefly, the membrane was prehybridized for 30 min at 65°C in 10 ml of Expresshyb solution. Hybridization was done at 65°C overnight with a cDNA probe mix that included 30 μg of Cot-1 DNA and 150 μg of sheared salmon testes DNA. The blot was washed 5 times in 2× SSC (0.3 M NaCl, 0.03 M sodium citrate) with 1% SDS at 65°C for 20 min followed by 20 min washes in 0.1× SSC with 0.5% SDS at 55°C for 20 min. The dot blot was exposed to x-ray film at −70°C for 48 h with an intensifying screen.

In Situ Hybridization—Mouse embryos (NIH Swiss, Harlan Sprague-Dawley, Indianapolis, IN) were used. The morning of plug exposure to x-ray film at

**Fig. 1. Kinetic characteristics of RDH1.** RDH and HSD activities were measured with all-trans-retinol (open circles) or 9-cis-retinol (filled circles) for 10 min with 5 μg of protein and 2 mM NAD⁺ (upper panel) or with 3-adiol (open circles) or androsterone (filled circles) for 3 min with 1 μg of protein and 2 mM NAD⁺ (lower panel). Representative data are shown from one of two experiments with the 800× g supernatant of transfected CHO cells.

**Fig. 3. RDH1 substrate recognition.** Substrate screening was done with 800× g supernatants of transfected CHO-K1 cells. A, activity with all-trans-retinol (striped bars) or 9-cis-retinol (solid bars). Conditions were as follows. 1, pcDNA3 (mock transfection); 2, pcDNA3/RoDH1 (rat RoDH1); 3–5, pcDNA3/mRDH1. Cofactors used were as follows. 1–3, 2 mM NAD⁺; 4, 2 mM NAD⁺; 5, none. Assays were done for 15 min with 50 μg of protein and 5 μM substrate. B, steroid dehydrogenase characteristics of RDH1 are as follows. I, 3-adiol (two products were observed, the more abundant was DHT; the less abundant was androstane-dione); 2, androsterone; 3, DHT; 4, testosterone; 5, estradiol; 6, corticosterone. Assays were done for 5 min with 2 μg of protein, 2 μM substrate, and 2 mM NAD⁺.

**Fig. 5. Biosynthesis of atRA from retinol by RDH1 and RALDH in intact cells.** CHO-K1 cells were transfected with fixed amounts of pcDNA3/RDH1 and graded amounts of mouse ALDH6 (circles; filled and unfilled represent data from two different transfections), mouse RALDH2 (open squares), or mouse AH2D (filled squares). Transfections with pcDNA3 or pcDNA/RDH1 alone or with any ALDH alone produced no detectable RA upon addition of retinol to the medium. Data show RA produced from 1 μM retinol (means ± S.D. of triplicate plates).
constructs nor any of the fragments was found in any public data base.

To determine whether additional all-trans-retinol recognizing SDR occur, five primer pairs (F1/R1; F1/R7; F10/R1; F11/R7) were used independently for reverse transcription-PCR with e17 mouse embryo mRNA. Note that although the PCR conditions were not varied, two different degenerate forward primers, F1 and F11, were designed from the “signature” SDR motif (GXWGXXVNNAG). In addition, two different degenerate reverse primers, R1 and R7, were designed from the same RoDH/CRAD motif (PRTXYSXGW). A third forward primer, F10, was designed from the SDR cofactor binding motif (FIT-GCDSGFG). Seventy-three of the clones generated were sequenced, 13–16 produced by each primer pair. Thirty-seven of the 73 clones sequenced encoded SDR. Only three encoded RDH1. Twenty-one encoded CRAD2; two encoded 17β-HSD9; two encoded RDH4; five encoded two previously unknown SDR with no activity for retinol; two encoded a previously unknown enzyme, CRAD3, with activity for 9- and 11-cis-retinol but little or no activity for all-trans-retinol. To probe further for additional RDH candidates, nested PCR was done with mRNA from e7 and e11 embryos, embryonic heart, adult testis, and adult small intestine. The first round was done with the primer mixture F10 (designed from the SDR cofactor binding motif), R1 and R9. The two degenerate reverse primers, R1 and R9, were designed from the CXXHALTX sequence conserved in RoDH/CRAD. The second round was done with the mixture of degenerate primers: F10, F11, R8, and R9. Sixty-seven of the clones produced were sequenced. RDH1 was amplified from e7 embryo (5 of 30 clones sequenced), e11 embryo (4 of 10 clones sequenced), and testis (2 of 10 clones sequenced). RDH1 was not amplified from embryonic heart or from adult small intestine. RDH4, a 9- and 11-cis-retinol-metabolizing SDR, accounted for 22 of the 30 clones sequenced from e7 mouse, 5 of the 10 sequenced from e11 mice, and all 10 sequenced from embryonic heart. The remaining clones sequenced encoded CRAD2, CRAD3, and 17β-HSD9. No other SDR were found that were active with all-trans-retinol.

Deduced Amino Acid Sequence—The protein deduced from pcDNA3/RDH1 has a calculated molecular mass of ~36 kDa and includes 6 peptides with high sequence similarity (73–100%) to the 6 motifs characteristic of SDR (26). Nineteen of the 23 amino acids conserved in ~70% of SDR occur within these 6 motifs. These motifs include the conserved cofactor binding region, G(3)X(3)GXD, and catalytic residues S(164)X(3)SX(1)K (Fig. 2). The deduced amino acid sequence also shares similarity with other members of the RoDH/CRAD subfamily of SDR but less similarity with PR-RDH, retSDR, 3β-HSD, and 11β-HSD (Table I). RDH1 has an 18-amino acid hydrophobic sequence at its N terminus bounded by four hydrophilic amino acids, R(10)ERQ, in common with other SDR that metabolize retinoids/steroids (17). This sequence targets the enzymes to the smooth endoplasmic reticulum and is essential for enzymatic activity.4

Enzymatic Activity—The supernatant from RDH1-transfected CHO cells was screened for enzyme activity with arbitrarily chosen amounts of protein and time. Under these conditions, the supernatant generated 745 ± 90 pmol of all-trans-retinol, 292 ± 6 pmol of 9-cis-retinal (mean ± S.D., n = 3) (Fig. 3), and no detectable 13-cis-retinal (not shown) from 5 μM all-trans, 9-cis-, or 13-cis-retinol and 2 mM NAD+, respectively. RDH1 had 8–11-fold higher activity with NAD+ versus NADP+ and had greater activity with all-trans-retinol than rat RoDH1, assuming equivalent transfection efficiencies. Both mouse RDH1 and rat RoDH1 had higher activity with all-trans-retinol versus 9-cis-retinol, but RoDH1 showed greater discrimination. RDH1 reached maximum activity at pH 8 and retained the same activity at pH values of 8.5 and 9. Activity at pH 7.5 was 54% that at pH 8 (data not shown). The most active steroid substrate tested was 3-adiol. RDH1 first converted 3-adiol via 3-adiol NADP+ (33 pmol), androstanedione (35 ± 3 pmol), and estrone (26 ± 6 pmol), respectively. 11β-HSD activity with corticosterone was detectable, but low, producing dehydrocorticosterone (19 ± 2 pmol).

Kinetic values for the four most actively metabolized sub-

3 R. Zhuang and J. L. Napoli, submitted for publication.

4 J. Wang and J. L. Napoli, submitted for publication.
strates were obtained under linear conditions of protein and time. The average $K_m$ value with all-trans-retinol was 2.6 μM (2.6 ± 0.1 and 2.7 ± 0.2, means ± S.E. of nonlinear regression analysis of two transfections) and with 9-cis-retinol was 2.2 μM (2.5 ± 0.3 and 1.8 ± 0.3). The average $V_m$ values were 6.7 (7 ± 0.1 and 6.4 ± 0.2) and 3.3 (3.5 ± 0.1 and 3.1 ± 0.1) nmol/min/mg of protein, respectively for all-trans- and 9-cis-retinal production (Fig. 4, upper panel). The average $K_m$ values for 3α-adiol and androsterone were 3.1 (2.3 ± 0.3 and 4 ± 1.3) and 3.5 μM (2.2 ± 0.3 and 4.8 ± 1), respectively, with average $V_m$ values of 97 (109 ± 4.6 and 86 ± 10) and 66 (56 ± 2.5 and 74 ± 6) nmol/min/mg of protein, respectively (Fig. 4, lower panel). The reaction with DHT was not saturated kinetically with substrate concentrations to 55 μM. These data indicate RDH1 efficiencies ($V_m/K_m$) of 31 (3-adiol), 19 (androsterone), 3 (all-trans-retinol) and 1.5 (9-cis-retinol) (nmol/min/mg)/μM.

**RA Synthesis in Intact Cells**—To determine whether RDH1 contributes to RA biosynthesis in intact cells, cotransfections were done with RDH1 and three mouse RALDH isozymes, AHD2, RALDH2, and ALDH6 (27–32). Mock transfections with pcDNA3 or transfection with RDH1 alone or with any one of the RALDH cDNAs alone produced no detectable RA from all-trans-retinol (data not shown). Combination of RDH1 with any one of the RALDH isozymes generated atRA from all-trans-retinol (Fig. 5). RA biosynthesis increased with increasing amounts of transfected DNA; small increases in vector amounts resulted in substantial increases in RA biosynthesis. Mouse RALDH2 seemed somewhat more efficient than the mouse ortholog of human ALDH6, whereas AHD2 was less efficient. Consistent with its activity in whole cells, purified recombinant RALDH1/AHD2 functions about 20% as efficiently ($V_m/K_{0.5}$) as RALDH2 in vitro (11). Thus, these results reflect the $V_m/K_{0.5}$ values of the three RALDHs (relative values of 100, 70, and 20 for RALDH2, ALDH6, and AHD2, respectively), demonstrating the predictive potential of RALDH enzymology in vitro (11, 32) and suggesting that the activity differences shown here did not stem from transfection efficiency differences.

**mRNA Expression**—Northern blotting revealed a major ~4.2-kb transcript in mouse liver and kidney 24 h after expo-
Mouse Retinol Dehydrogenase Type 1 (RDH1)

Characteristics of mouse SDR that metabolize retinoids and steroids

| Mouse enzyme (gene) | Retinoids | Substrate specificities | Steroids | mRNA loci |
|---------------------|-----------|------------------------|----------|-----------|
| RDH1 (Rdh1)         | All-trans-retinol > 9-cis-retinol | 3α-HSD (3-adiol > androstenedione) > 17β-HSD (DHT > testosterone > estradiol) | Many (see text under mRNA expression) | Liver > kidney > intestine > others |
| CRAD1 (Rdh6)        | 9-cis-retinol = 11-cis-retinol > all-trans-retinol | 3α-HSD (3-adiol > androstenedione); little or no activity | Liver > lung > eye > kidney |
| CRAD2 (Rdh7)        | 11-cis-retinol >> 9-cis-retinol (testosterone) | 17β-HSD activity | Many; liver >> kidney >> others |
| CRAD3               | 9-cis-retinol = 11-cis-retinol >> all-trans-retinol | 3α-HSD (3-adiol > androstenedione); little or no activity | Liver > kidney > brain |
| RDH4 (Rdh4)         | 9-cis-retinol = 11-cis-retinol >> all-trans-retinol | 17β-HSD activity | Pancreas > liver > kidney > retina |
| retSDR1             | All-trans-retinol reductase | None detected | |
| 17βHSD9 (Rdh8)      | All-trans-retinol (~20-fold < active than RDH1) 11-cis-retinol >> 9-cis-retinol | 17β-HSD (estradiol > DHT > testosterone) > 3α-HSD (3-adiol = androstenedione) | Liver |

Dot blot analysis with an RNA master blot confirmed and extended the Northern blot data (Fig. 7). RDH1 mRNA expression was detected in all Northern tissues examined. Expression was intense in pancreas, testis, skeletal muscle, brain, heart, submaxillary gland, liver, and kidney. Expression was detected in eye, lung, smooth muscle, thyroid, thymus, ovary, prostate, epididymis, and uterus. E7, e11, e15, and e17 mice expressed RDH1, in agreement with the Northern blot data.

Cellular mRNA Expression Patterns—In situ hybridization results were consistent with the Northern and dot blot data (Fig. 8). RDH1 transcripts were most abundant during stages of early organogenesis in the mouse, from e7.5 to e10.5. RDH1 mRNA was expressed throughout the embryo and extraembryonic regions, with particular enrichment within the neural plate at e7.5 and within the neural tube, gut, neural crest, and Rathke’s pouch at e10.5. By e14.5, RDH1 expression reflected its adult pattern. Transcripts were abundant in the developing eye, ventral neural regions, cartilage, liver, and lung but appeared relatively less intense elsewhere. Sense controls were done at all stages, and all gave background signals.

Concluding Summary—This report identifies the first all-trans-retinol RDH1/SDR candidate in the mouse and suggests that mouse and rat may differ in the initial step of RA biosynthesis from retinol. An enzyme physiologically significant in atRA biosynthesis should be expressed by all vertebrates and should be well conserved among orthologs. Attempts to identify mouse SDR candidates for atRA biosynthesis, however, have not identified all-trans-retinol-favoring enzymes and instead have revealed SDR more efficient with cis-retinols than all-trans-retinol, including CRAD1, -2, -3 and -4 (18, 19, 21). Failure to identify mouse RDH with preferential activity for all-trans-retinol cast doubt on the universal importance of SDR to RA biosynthesis. Identifying an enzyme in the mouse that catalyzes retinol production from retinol represents a crucial step to understanding atRA biosynthesis, provides opportunity to take advantage of the studied embryonic development of the mouse, and provides a convenient knock-out opportunity.

No other efficient all-trans-RDH have been identified in mouse either here in our analysis of multiple clones produced from diverse templates with different sets of redundant primers or in previous work, and no public data base includes mouse RDH1 or portions thereof. Yet multiple cis-retinoid RDH and retinol reductases have been identified (Table II). Mouse 17β-HSD9 has weak all-trans-retinol RDH activity, ~5% of RDH1, but as its name implies, is closer phylogenetically to 17β-HSD than to RDH. In contrast to 17β-HSD9, RDH have little or no detectable 17β-HSD activity. Three rat RoDH SDR isozymes have been identified that manifest their most efficient RDH activity with all-trans-retinol, and their cDNAs have been cloned (34–36). Two, RoDH1 and RoDH2, show widespread mRNA, but only liver expresses the third, RoDH3. These were the first SDR identified as candidates for contributing to the physiological path of RA biogenesis. Mouse RDH1 differs from the rat enzymes in several respects. Three closely related rat enzymes occur with amino acid similarities between 88 and 99%. The rat enzymes were detected by Northern blot only in liver and were detected in extra-hepatic tissues with RNase protection assays or in situ hybridization (34–36, 37), in contrast to the widespread mRNA expression of RDH1 detectable by Northern blot. Potential human homologues also have been cloned using probes from RoDH1 (38, 39). A human SDR, hRDH-E, shares ~80% amino acid similarity with the three rat isozymes but has expression limited to liver and epidermis. A second putative human RoDH shares less amino acid similarity with the rat enzymes (~72%) and may function primarily in steroid metabolism (33, 40). So far only RDH-E and the RoDH-like HSD have been identified in human. Future work should clarify RDH that are oothologous in rat, mouse, and human and should confirm whether additional RDHs occur in mouse.

Factors consistent with RDH1 functioning to biosynthesize atRA include its expression during early embryogenesis and in all known vitamin A target tissues probed and its ability to provide retinal to each of theRALDH isozymes for the biosynthesis of atRA in intact cells.
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