We report a mouse short-chain dehydrogenase/reductase (SDR), retinol dehydrogenase-similar (RDH-S), with intense mRNA expression in liver and kidney. The RDH-S gene localizes to chromosome 10D3 with the SDR subfamily that catalyzes metabolism of retinoids and 3α-hydroxysteroids. RDH-S has no activity with prototypical retinoid/steroid substrates, despite 92% amino acid similarity to mouse RDH1. This afforded the opportunity to analyze for functions of non-catalytic SDR residues. We produced RDH-SΔ3 by mutating RDH-S to remove an "additional" Asn residue relative to RDH1 in its center, to convert three residues into RDH1 residues (L121P, S122N, and Q123E), and to substitute RDH1 sequence G208FKTCVTSSD for RDH-S sequence F208FLTGMASSA into RDH1 produced reactivity to analyze for functions of non-catalytic SDR residues. We produced RDH-SΔ3 by mutating RDH-S to remove an "additional" Asn residue relative to RDH1 in its center, to convert three residues into RDH1 residues (L121P, S122N, and Q123E), and to substitute RDH1 sequence G208FKTCVTSSD for RDH-S sequence F208FLTGMASSA into RDH1 produced "viz."

The short-chain dehydrogenase/reductase (SDR) family encodes ~100 bacterial, plant, and animal members related through a limited number of conserved residues that determine structure, provide for cofactor binding, and catalyze dehydrogenation/reduction (1–3). SDR family members do not always share substantial amino acid identities and have relatively few strictly conserved residues. Animal SDR catalyze intermediary metabolism and activation/inactivation of nuclear receptor ligands such as prostaglandins, retinoids, and steroid hormones. SDR tend to have multifunctional catalytic abilities: they can catalyze reactions with dissimilar substrates and/or can recognize functional groups in different loci of the same substrates. An apparent subgroup of the SDR superfamily consisting of phylogenetically related enzymes catalyzes dehydrogenation of all-trans-retinol, cis-retinols, and androgens or reduction of retinals. Functions of this subgroup could include serving in the visual cycle, generating the endocrine factors all-trans-retinoic acid and 9-cis-retinoic acid, reducing retinal produced by carotenoid metabolism, and/or reactivating 5α-androstan-3α,17β-diol (3α-adiol) into dihydrotestosterone (4, 5).

Of the mouse SDR in the retinoid subfamily, RDH1 has widespread expression and seems to have the highest catalytic efficiency for all-trans-retinol dehydrogenation; mouse 17β-HSD9 catalyzes all-trans-retinol dehydrogenation about an order of magnitude less efficiently than RDH1, and RNase protection assays reveal 17β-HSD9 mRNA expression only in liver (6, 7). Other mouse SDR such as CRAD1 and CRAD3 catalyze 9-cis-retinol dehydrogenation much more efficiently than all-trans-retinol dehydrogenation and have weak, if any, activity with all-trans-retinol (8, 9). CRAD2 has very low efficiency for all-trans-retinol and even lower efficiency for 9-cis-retinol (10). RDH4 catalyzes all-trans-retinol dehydrogenation at least 2 orders of magnitude less efficiently than RDH1 (11, 12). The mouse SDR retSDR1, RRD, and P5SDR1 function as reductases that convert all-trans-retinol into all-trans-retinol, but do not catalyze dehydrogenation of all-trans-retinol (13–16). Therefore, although an array of SDR catalyze retinoid metabolism, RDH1 represents the only SDR thus far in the mouse with high catalytic efficiency for all-trans-retinol dehydrogenation and widespread tissue expression, with expression initiating early during embryogenesis.

Here we report cDNA cloning of a novel mouse SDR gene family, provide insight into the function of non-catalytic SDR residues, and illustrate that limited changes in the multifunctional SDR yield major alterations in substrate specificity and/or catalytic efficiency.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AJ046408.

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The abbreviations used are: SDR, short-chain dehydrogenase(s)/reductase(s); 3α-adiol, 5α-androstan-3α,17β-diol; HSD, hydroxysteroid dehydrogenase; CRAD, cis-retinol/androgen dehydrogenase; RDH, retinol dehydrogenase(s); RDH-S, retinol dehydrogenase-similar; mRDH, mouse retinol dehydrogenase; rRDH, rat retinol dehydrogenase; RDH-E, retinol dehydrogenase-epidermal; RACE, rapid amplification of cDNA ends; E17, embryonic day 17.
cDNA Cloning of Mouse RDH-S (mRDH-S)—Mouse E17 poly(A)

DNA (Sigma) was subjected to reverse transcription at 42 °C for 60 min using random hexamers. PCR was done with primers 5'-TTTATAC-

GNGGNTGYGAYTCNGGNTTYGGN (forward) and 5'-R5 (CTGACATGATGGTCTACATTGTAG) using mouse e17 cDNA. This PCR product was

used as template with sense primer 5'-CCCGGAATTCGCCGCTAAGAGCGAACACTGTATCAGTGAGTAACTTGTAACCACACAGAGCTATGATAAACATGACGTCAGAGGGCTTTCTCA; underlining indicates a Kozak sequence) and antisense primer 5'-GGCGGCTTCAGCAACAAACACTTG-3' (reverse); C212G, 5'-TTCAAGACTGGCGTGACAAGT-3' (reverse); T214A, 5'-ACTTGTCACGCCAGTCTT-3' (forward) and 5'-TCCAAGACTGGCGTGACAACATGACGTCAGAGGGCTTTCTCA; underlining indicates an Xhol site). The PCR product was gel-purified, digested with EcoRI/ Xhol, and cloned into plasmidp to construct pcDNA3/RDH-S.

Radiation Hybrid Mapping—A mouse radiation hybrid panel was purchased from Research Genetics. A forward primer (CCCTGTTGAG-

GAGG) and R4 (5'-CCGCTGATTCGAGGGCTTTCTCA; underlining indicates a Kozak sequence) and antisense primer R6 (5'-CCGCTGATTCGAGGGCTTTCTCA; underlining indicates an Xhol site). The PCR product was gel-purified, digested with EcoRI/ Xhol, and cloned into plasmidp to construct pcDNA3/RDH-S.

Northern Blotting—A probe from the 3'-untranslated region of mRDH-S was amplified from the cDNA with the primers used for radiation hybrid mapping. The PCR product was cloned into vector pGEM-T, sequenced, and labeled with [32P]dCTP using the RadPrime DNA labeling system (Invitrogen). The probe was hybridized overnight at 68 °C with a mouse multiple-tissue blot (Clontech) according to the manufacturer's protocol. Mouse β-actin cDNA was used as a control. Blots were exposed to x-ray film with an intensifying screen at −70 °C for 1 day.

Mouse RDH1 (mRDH1) Mutants—mRDH-S and mRDH1 cDNAs were amplified using forward primer 5'-CGGGATCCATGCGGCT-

GCTTAACCT-3' with an engineered BamHI restriction site and a Kozak sequence, and reverse primer 5'-CCGCGCTTCAGCAACCATGACGTCAGAGGGCTTTCTCA; containing an EcoRI site. PCR products were digested with BamHI and EcoRI and cloned into pcDNA3. We used restriction sites to create chimeras/mutants A7P, C1, C2, C2A, and C3. Chimeras C3 and C5 were made by PCR mutagenesis. Sequences were confirmed by DNA sequencing.

Site-directed Mutagenesis—Mutagenesis was done using the BamHI/ XhoI sites of pcDNA3/mRDH1. Splicing by overlap extension was used in PCR amplification with Pf loop polymers of an mRDH1 region with primers containing the desired mutation (17). The primers were 18 or 21 nucleotides long with the mutation located centrally: K210E, 5'-AACACATGTGGTCTACTACATGACGTCAGAGGGCTTTCTCA; containing an EcoRI site. PCR products were digested with BamHI and EcoRI and cloned into pcDNA3. We used restriction sites to create chimeras/mutants A7P, C1, C2, C2A, and C3. Chimeras C3 and C5 were made by PCR mutagenesis. Sequences were confirmed by DNA sequencing.

2 Available at www.genome.wi.mit.edu/cgi-bin/mouse_rh/rhmap-auto/rhmapper.cgi.
cin. Cells were transfected with pcDNA3 constructs (8% supplemented with 10% fetal calf serum and 1% penicillin/streptomycin. Analyses of 3H-labeled steroids (20,000 dpm/reaction) were performed using liquid scintillation counting as described (6).

Expression of RDH—CHO-K1 cells were transfected with pcDNA3 or pFLAGCMV5a (Sigma) served as controls. Protein concentrations were determined by the dye-binding method (18).

Molecular Modeling

Mouse RRD 42 16 28 AB045132
Human pr-RDH 44 19 33 AF229845
Human retSDR1 44 21 32 AF061741
Mouse retSDR1 45 22 32 AF061743
Mouse CRAD1 (Rdh6) 100 100 100 AY046408
Rat RoDH2 90 85 90 U33500
Rat RoDH1 85 79 86 U33501
Rat RoDH1 85 79 86 U18762
Human RDH-E/RDH4 78 71
Human HSD/RDH-TBE 71
Mouse 17β-HSD9 (Rdh8) 70
Rat 17β-HSD6 70
Mouse SDR-O 63
Mouse 9,11-cis-RDH (Rdh4) 60
Human 9,11-cis-RDH (Rdh5) 60
Human 3α-HSD/RDH-TBE 55
Mouse retSDR1 45
Human retSDR1 44
Human pr-RDH 44
Mouse RRD 42

| Species | SDR (gene) | Nucleotide identity | Amino acid homology | GenBank/EBI accession no. |
|---------|------------|---------------------|--------------------|--------------------------|
| Mouse   | RDH-S      | 100                 | 100                | Y406408                  |
| Mouse   | CRAD1 (Rdh8) | 93               | 91                 | AF020513                  |
| Mouse   | CRAD3 (Rdh9) | 93               | 90                 | AF372838                  |
| Mouse   | RDH1 (Rdh1) | 92                | 89                 | AY28928                   |
| Rat     | RoDH2      | 90                 | 85                 | U33500                    |
| Rat     | RoDH1      | 85                 | 80                 | AF56194                   |
| Rat     | RoDH1      | 85                 | 80                 | U33501                    |
| Human   | RDH-E/RDH4 | 78                 | 70                 | NM003708/AF086735         |
| Human   | HSD/RDH    | 71                 | 65                 | U59281/AF223225           |
| Mouse   | 17β-HSD9   | 70                 | 66                 | AF103797                  |
| Mouse   | SDR-O      | 63                 | 63                 | U89290                    |
| Mouse   | 9,11-cis-RDH (Rdh4) | 60 | 51                 | AY04434                  |
| Human   | 9,11-cis-RDH (Rdh5) | 60 | 50                 | AF013288                  |
| Human   | 3α-HSD/RDH-TBE | 55 | 47                 | U43559                    |
| Mouse   | retSDR1    | 45                 | 47                 | AF43279/AY017349          |
| Human   | retSDR1    | 44                 | 22                 | AF061743                  |
| Human   | pr-RDH     | 44                 | 21                 | AF029845                  |
| Mouse   | RRD        | 42                 | 16                 | AB045132                  |

RESULTS AND DISCUSSION
cDNA Cloning of RDH-S—We sequenced 14 clones encoding SDR after reverse transcription-PCR of mouse E17 embryo mRNA using primers from conserved regions (R3′TGGCDSFGG and S3′K/Y/F/G/(I/V/L/F/M)/EAPSDF) of retinoid/steroid-metabolizing SDR. Eight encoded CRAD2 (10); two encoded 17β-HSD9 (7); three encoded an orphan SDR (22); and one encoded a novel SDR, which we named RDH-S. 5′- and 3′-RACE generated the full-length coding sequence of mRDH-S. The 3′-untranslated region was extended further with two rounds of 3′-RACE to generate a cDNA of 1890 bp (Fig. 1). The open reading frame encodes a deduced protein of 318 amino acid residues with high homology to other SDR that catalyze retinoid/steroid metabolism (Table I). mRDH-S differs in only 33 residues from RDH1, many of them conservative substitutions (Fig. 2). Notably, RDH-S has the six peptide motifs characteristic of retinoid/steroid-metabolizing SDR (1). Nineteen of the 23 amino acid residues conserved in ~70% of SDR occur within these six motifs. Conserved residues include the cofactor-binding sequences T[G/X]G,XGX and N111NAG in the first and second motifs, the catalytic sequence S150X11,YX,X in the fourth and fifth motifs, part of the substrate-binding domain in the sixth motif, and the oligomerization domain in the third motif. An additional amino acid, Asn, occurs in RDH-S between residues 173 and 174 (RDH1 numbering), which potentially could change the secondary/tertiary structure relationship between catalytic residues Ser164, Tyr176, and Lys180.

RDH-S mRNA Expression—Northern blot analysis was done with a 3′-untranslated region probe. mRDH-S mRNA was intensely expressed in liver and kidney, but expression was not observed in the six other tissues analyzed (Fig. 3). Four sizes of
mRNA occurred in liver, with the major ones at 2.4, 3.4, and 4.4 kb. The 3.4-kb mRNA was not expressed in kidney.

**RDH-S Chromosomal Localization**—Radiation hybrid mapping indicated that mRDH-S locates to mouse chromosome 10D3 between markers D10Mit269 and D10Mit271 and near CRAD1 (Rdh6) and CRAD2 (Rdh7). The RDH-S gene maps close to seven other members of the subfamily (Fig. 4).

**Lack of mRDH-S Enzyme Activity**—mRDH-S, which shares 92% amino acid similarity and 89% identity with mRDH1 (Fig. 2 and Table I), did not catalyze metabolism of the major substrates recognized by mRDH1. These include all-trans-retinol, 9-cis-retinol, 3/H9251-adiol, and androsterone (data not shown). The lack of RDH-S activity was surprising because none of the residue differences occurred in obviously crucial sections (see above), and many were conservative substitutions. These limited differences between RDH1 and RDH-S afforded the opportunity to distinguish the residues in SDR that contribute to activity with retinoids and steroids.

**Distinct Requirements for Steroid Versus Retinoid Activity**—The non-conservative differences provide obvious starting points for evaluating the effects of substituting RDH-S residues into RDH1. The RDH1 mutant A7P behaved enzymatically similar to RDH1; therefore, no further analysis was done with this mutant. Substituting the section of RDH-S from residues 117 to 147 into RDH1 produced chimera C1, with nine total and eight non-conservative residue differences from RDH1 (Figs. 2 and 5). C1 had no detectable activity with all-trans-retinol or 3/H9251-adiol (Fig. 6, A and B, bars 4). C1 mutant L121P/S122N/Q123E, i.e. C1/PNE, was made because sequence L121SQ in C1 differs substantially from P 121NE conserved in mRDH1, rat RoDH1–3, and the human ortholog RDH-E. C1/PNE had partial activity with all-trans-retinol and 3-adiol (Fig. 6, A and B, bars 5). Mutating any two of the three C1 residues produced chimera mutants without activity (C1/PN, C1/PE, and C1/NE) (Fig. 6, A and B, bars 6–8). Chimera C2 differs from RDH1 in four residues, with one non-conservative change. C2 had par-
tial activity with both substrates (Fig. 6, A and B, bars 9). Chimera C2 with Asn inserted between residues 173 and 174 of RDH1, i.e. C2+N, was inactive with both substrates (Fig. 6, A and B, bars 10). Chimera C3 differs in six residues from mRDH1, with three non-conservative changes. C3 was not active with all-trans-retinol, but catalyzed 3α-adiol metabolism at a higher rate than RDH1 (Fig. 6, A and B, bars 11). Chimera C4, which includes the RDH-S residues of C3 as well as six additional differences, four of which are non-conservative, was inactive with both all-trans-retinol and 3α-adiol (Fig. 6, A and B, bars 12). Consequently, only all-trans-retinol activity requires the specific C3 area residues of RDH1, but 3α-adiol activity requires the residues in the C-terminal part of C4, i.e. Lys224, Lys229, and Ala230. Chimera C5, which partially over-
of RDH1 into RDH-SΔ3 created a catalytically active protein with lower \( K_m \) values compared with those of RDH1 with both all-trans-retinol and 3α-adiol, albeit one less efficient than RDH1 because of lower \( V_{max} \) values. With the exception of RDH-SΔ3, the mutations affected activity with all-trans-retinol and 3α-adiol much differentially. C1/PNE showed major (−10-fold or greater) increases in \( K_m \) values for both all-trans-retinol and 3α-adiol, but maintained an efficient \( k_{cat} \) only with all-trans-retinol. C9 had no detectable activity with all-trans-retinol, but had ∼4-fold higher efficiency with 3α-adiol compared with RDH1, predominantly because of a 5-fold lower \( K_m \) value. C5 showed substantial efficiency with all-trans-retinol, but was unsaturated kinetically with 3α-adiol. The activity of C6 with all-trans-retinol was only 20% as efficient as that of RDH1, despite a \( V_{max} \) value 4-fold higher, but was not saturated kinetically with 3α-adiol. These results seem remarkable because most of the residue differences in each construct are conservative relative to RDH1, consistent with a large impact of a very few non-conservative changes.

**RDH1 Residues 208–217 Contribute to Retinol Specificity**—C3 was assayed with 9-cis-retinol and androsterone because mRDH1 catalyzes metabolism of both (6). C3 had weak activity with 9-cis-retinol, just as it did with all-trans-retinol, but substantial activity with androsterone, just like with 3α-adiol, which reinforces the conclusion that retinoid recognition requires the specific C3 residues of RDH1. This conclusion is also supported by the mutations in C3, which has no detectable activity with all-trans-retinol, but had ∼20% lower \( V_{max} \) values. C3 also had no detectable activity with 3α-adiol, but had ∼5-fold lower \( k_{cat} \) value. C5 showed substantial efficiency with all-trans-retinol, but was not detectable with 3α-adiol, and C6 was unsaturated kinetically with 3α-adiol. These results seem remarkable because most of the residue differences in each construct are conservative relative to RDH1, consistent with a large impact of these residues due to the high efficiency with 3α-adiol.
ent with the tolerance observed here in the D217A mutant. Mutants V213M and T214A had reduced efficiencies stemming from increased $K_m$ values, despite a 3-fold increase in the $V_m$ value in the case of V213M. Most SDR with measurable retinol dehydrogenase activity have Val213 (RDH1, rRoDH1–3, mouse/human RDH4/5, and human RDH-E); 17β-HSD9 provided the exception, with a Met residue like that of RDH-S, but 17β-HSD9 showed much lower efficiency than RDH1. Each SDR with measurable retinol dehydrogenase activity has a Thr residue at position 214, including 17β-HSD9, consistent with the noted negative effect of the T214A mutation. These data and activity comparisons among the retinoid dehydrogenase SDR indicate that loss of all-trans-retinol-metabolizing activity with chimera C3 could have been caused by K210L alone, but probably was exacerbated by V213M and T214A.

Differences in Expression Are Not Responsible for Differences in Activities—Western blotting confirmed the expression of those of RDH1 (Fig. 8, lower panel). These data exclude protein expression differences as a major contributor to changes in $V_m$ values.

Molecular Modeling—We generated a molecular model based on three-dimensional structures of soluble SDR using an optimal amino acid sequence alignment. The model relied on x-ray structures of human 17β-HSD type I complexed with cofactor and/or substrate (39.8% identity; Protein Data Bank codes 1FDS, 1A27, and 1EQUB) (23–25), 20β-HSD (51.5%; Protein Data Bank code 1HU4) (26), and β-ketoacyl-(acyl-car-

### TABLE II

| RDH          | All-trans-retinol | 3α-Adiol |
|--------------|-------------------|----------|
|              | $K_m$ (μM) | $V_m$ (nmol/min/mg) | $V_m/K_m$ | $K_m$ (μM) | $V_m$ (nmol/min/mg) | $V_m/K_m$ |
| mRDH1        | 4.7 ± 2.2 | 2.4 ± 0.3 | 0.5 | 9 ± 0.9 | 50 ± 9 | 6 |
| RDH-S33      | 1.8 ± 0.6 | 0.5 ± 0.3 | 0.3 | 6.1 ± 0.1 | 26 ± 1.5 | 4 |
| C1/FNE       | 40 ± 9     | 2.2 ± 0.02 | 0.05 | >20b     | 43 ± 9 | 23 |
| C3           | ND         | ND        | ND | >20b     | 43 ± 9 | 23 |
| C5           | 3.4 ± 2.8  | 1.5 ± 0.1 | 0.4 | >20b     | 43 ± 9 | 23 |
| C6           | 64 ± 18    | 9.1 ± 1.7 | 0.1 | >20b     | 43 ± 9 | 23 |

| Mutation     | $K_m$ (μM) | $V_m$ (nmol/min/mg) | $V_m/K_m$ relative to RDH1 |
|--------------|------------|---------------------|-----------------------------|
| K210L        | >400       | <0.07               | <0.0002                     | <0.0004                     |
| C212G        | 5.4 ± 0.4  | 6.4 ± 0.1           | 1.2                         | 2.4                         |
| V213M        | 27 ± 0.6   | 6.2 ± 2.1           | 0.2                         | 0.4                         |
| T214A        | 23 ± 0.4   | 2.3 ± 0.02          | 0.1                         | 0.2                         |
| D217A        | 10 ± 4.2   | 4.8 ± 0.2           | 0.5                         | 1                           |

* ND, no activity detected (<0.02 nmol/min/mg of protein).

* Reactions were not saturated at 20 μM substrate.
Fig. 9. **Molecular model of mRDH1.** N indicates residue 28 at the N terminus, and C, shows the C-terminal residue. Green indicates backbone residues that were not the focus of this work. Black depicts the cofactor-binding residues Gly42, Gly44, and Gly46. Orange depicts the catalytic residues Ser164, Tyr176, and Lys180. Dark blue depicts chimera C1 with the P121NE sequence depicted in yellow. Yellow shows Gly173 and Gly174, which are interrupted by an Asn residue in RDH-S, indicated by an asterisk. Yellow also depicts chimera C3, with Lys224, Lys229, and Cys232 shown in yellow. Red depicts residues 224–242 of chimera C5. Cyan depicts residues 243–316, which compose chimera C6 and the latter residues of C5.

The model suggests that elimination of activity by the Asn insertion in RDH-S between Gly173 and Gly174 results from a change in the orientation of the catalytic residue Tyr176 and perhaps Lys180 and the relationship between the two. It also suggests that the α-helix and β-sheet in the area represented in chimera C1 secure the substrate near cofactor-binding residues (Gly42, Gly44, and Gly46) and catalytic residues (Ser164, Tyr176, and Lys180). The P121NE sequence in this area appears to be essential for alignment. The LSQ substitutions in chimera C1 and in RDH-S likely eliminate activity by changing the orientation of the internal β-sheet (P121L) and by altering substrate-binding (NE to SQ). The model reveals the juxtaposition of RDH1 residues 208–217 with the catalytic residues and suggests that the differences in this area in RDH-S, i.e. the residues of chimera C3, alter substrate alignment with the catalytic residues. Thus, creating RDH-SΔ3 from RDH-S likely produces enzyme activity by affecting substrate alignment with catalytic residues.

Lack of C4 activity reinforced the observations with C3 and C5 (C3 had extraordinary specificity and 4-fold enhanced efficiency for 3a-adiol, whereas C5 showed specificity for retinol) because C4 contains the mutations of C3 and the section of C5 that does not overlap with C6 (RDH-1 residues Lys224, Lys229, Ala230, Val234, and Lys235). A “stick” depiction of the two single mutations in the C3 region with the largest effects (K224I and K225G) indicated that their side chains do not protrude into the active site cavity (data not shown). Direct steric interactions in the active-site pocket therefore seem unlikely. Notably, SDR with substantial all-trans-retinol dehydrogenation activity (e.g. rRDH1; human RDH-E, and 17β-HSD9) have a basic residue (Lys or Arg) at position 210. Those with stronger cis-retinol activity (e.g. CRAD1 and CRAD3) have a Leu residue at position 210. These data show that the Lys residue may be necessary but not sufficient for all-trans-retinol dehydrogenase activity. The results with C5 seem more remarkable because the impact of the five residue differences in the section of C5 that does not overlap with C6 had to overcome the deleterious influence of the residue differences that C5 and C6 have in common. The two conservative differences in the C4 end of C5 (V223I and K225G) draw attention to the probable disproportional influence of the other three (K224I, K225G, and T226I) and indicate that these three residues preserve the all-trans-retinol activity of chimera C5. Examination of other retinoid/3a-adiol-metabolizing SDR confirms that retinol (but not 3a-adiol) activity tolerates these variations well. For example, CRAD3 with Gln224, Gln229, and Thr230 has a 3-fold decrease in efficiency with 3a-adiol relative to RDH1 (9). Overall, the model suggests that residues in the C4 end of C5 influence the orientation of the C3 residues and that the C3 residues affect catalytic residue/substrate orientation and perhaps substrate binding.

**Concluding Summary**—We have reported the occurrence of a new member of the mammalian SDR superfamily, mRDH-S. Characterization of RDH-S was instrumental in developing data to help define the function of SDR residues that contribute to the substrate-binding pocket. The data from contrasting RDH-S and RDH1 demonstrate that minor modifications to the multifunctional SDR can cause major changes in catalytic efficiency and/or substrate specificity. The function of RDH-S remains unknown. Possibly, RDH-S catalyzes metabolism of ligands for “orphan” nuclear receptors. The Asn insertion in RDH-S argues against this, however, because it would perturb the orientation of catalytic residues. RDH-S may have a regulatory function, i.e. its expression may regulate metabolism by binding potential substrates and/or products and/or by serving as a regulatory factor (dominant-negative). In addition, expression of the mRNA does not guarantee translation: the mRNA may have a regulatory function. We are investigating the latter possibility.

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