Characterization of Mouse Short-chain Aldehyde Reductase (SCALD), an Enzyme Regulated by Sterol Regulatory Element-binding Proteins*

Received for publication, May 12, 2003, and in revised form, June 13, 2003
Published, JBC Papers in Press, June 13, 2003, DOI 10.1074/jbc.M304969200

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Sterol regulatory element-binding proteins (SREBPs) enhance transcription of genes encoding all of the proteins required for the cellular synthesis and uptake of cholesterol and unsaturated fatty acids. Here, we use suppression subtractive hybridization to identify a previously unrecognized SREBP-enhanced gene in mice. The gene encodes a membrane-bound enzyme that we designate SCALD, for short-chain aldehyde reductase.

We expressed SCALD in bacteria, purified it extensively, and studied its catalytic properties in detergent solution. The enzyme specifically uses NADPH to reduce a variety of short-chain aldehydes, including nonanal and 4-hydroxy-2-nonenal. The enzyme also reduces retinaldehydes, showing equal activity for all-trans-retinal and 9-cis-retinal. Northern blot analysis indicates that SCALD is expressed most abundantly in mouse liver and tests. In the liver of mice, SCALD is suppressed by fasting and induced by refeeding, consistent with regulation by SREBPs. In tests, SCALD expression is restricted to pachytene spermatocytes, as revealed by visualization of mRNA and protein. SCALD is also expressed in four layers of the retina, including the outer segment of rods and cones, as revealed by immunohistochemistry. SCALD appears to be the mouse ortholog of the human protein that has been designated variously as prostate short-chain dehydrogenase/reductase 1, retinal reductase 1, and retinol dehydrogenase 11. In view of its ability to reduce short-chain aldehydes in addition to retinals, we propose that SCALD may be induced by SREBP in liver and other tissues to prevent toxicity from fatty aldehydes that are generated from oxidation of unsaturated fatty acids that are synthesized as a result of SREBP activity.

Sterol regulatory element-binding proteins (SREBPs) are transcription factors that activate at least 20 target genes involved in cholesterol and unsaturated fatty acids biosynthesis (1–3). The SREBPs differ from other transcription factors because they are synthesized as membrane-bound proteins, the active fragments of which must be released by proteolysis to enter the nucleus and activate transcription. The proteolytic release is controlled by the lipid content of the cells; release is rapid when cells are depleted of cholesterol, and it is blocked when cholesterol overaccumulates (4, 5).

Mammalian cells express three isoforms of SREBP. SREBP-1a and SREBP-1c are produced from a single gene through the use of alternate transcription start sites, and SREBP-2 is encoded by a separate gene (6). In vitro, SREBP-1c, and SREBP-2 are the predominant isoforms, and their physiological roles have been studied in transgenic mice overexpressing the cleaved nuclear form of SREBPs (nSREBPs) in liver (1). In these mice, nSREBP-1c preferentially enhances transcription of genes required for fatty acid and triglyceride synthesis: acetyl-CoA carboxylase, fatty-acid synthetase, long-chain fatty-acyl elongase, and stearoyl-CoA desaturase. nSREBP-2 preferentially increases the level of mRNAs encoding multiple enzymes in the cholesterol biosynthetic pathway, including 3-hydroxy-3-methylglutaryl coenzyme A, 3-hydroxy-3-methylglutaryl coenzyme A reductase, farnesyl-diphosphate synthase, squelene synthase, lanosterol demethylase, and others. As a result of these changes, there is a massive increase in the content of unsaturated fatty acids and cholesterol in livers of transgenic mice overexpressing nSREBPs (1).

In the current study, we used the technique of suppression subtractive hybridization (7) to identify additional genes whose mRNAs are regulated by SREBPs (8). Specifically, we compared the expression levels of mRNAs from two sources of brown adipose tissue, one from wild-type mice and the other from transgenic mice overexpressing nSREBP-1c in adipose tissue (9). Among the most differentially expressed genes, we found a nucleotide sequence encoding a protein, hereafter referred to as short-chain aldehyde reductase (SCALD), that is regulated by all three SREBP isoforms.

SCALD is the mouse ortholog of a human protein that has already been given three names: prostate short-chain dehydrogenase/reductase 1 (PDSR1) (10), retinal reductase 1 (RalR1) (11), and retinol dehydrogenase 11 (RDH11) (12). The human mRNA was discovered as a gene that is expressed at very high levels in human prostate (10) and at lower levels in a variety of other human organs (12). The mRNA was induced 3-fold when

§§ This work was supported by Grant HL20948 from the National Institutes of Health and grants from the Perot Family Foundation, Moss Heart Foundation, and W. M. Keck Foundation.
The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF474027.
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***The abbreviations used are: SREBP, sterol regulatory element-binding protein; nSREBP, nuclear form of SREBP; SCALD, short-chain...
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prostate adenocarcinoma cells were treated with androgen. The cDNA sequence predicted that the protein was an enzyme of the family of short-chain aldehyde reductases, hence the original name PSDR1. The protein was overexpressed in insect tissue culture cells by cDNA transfection, and this led to an increase in the activity of a membrane-bound enzyme that reduced all-trans-retinal to the corresponding alcohol using NADPH as an electron donor (11). The enzyme also reduced 9-cis-retinal and 13-cis-retinal. This led to the renaming of the enzyme as RalR1. A subsequent study using histochemistry demonstrated that PSDR1 is expressed in human and bovine retinal pigment epithelium, and the enzyme was renamed RHDI (12). The previous publications on the human enzyme did not examine the ability of the enzyme to reduce any aldehydes other than retinoids.

To determine the substrate specificity of mouse SCALD, we produced the enzyme in bacteria, purified it to near homogeneity, and reconstituted it in active form. Here, we show that the enzyme has activity against short-chain aldehydes such as nonanal in addition to retinaldehydes. Polynsaturated fatty acids can give rise to a variety of aldehydes, including nonanal, when they undergo oxidation (13). The promiscuous specificity of SCALD stands in contrast to another aldehyde dehydrogenase (designated photoreceptor retinol dehydrogenase (prRDH) (14)) that we show is specific for retinaldehydes and does not act upon nonanal. Moreover, we show that SCALD is up-regulated by SREBPs, which also up-regulate the expression of the enzymes that synthesize and elongate unsaturated fatty acids (1). Unlike prRDH, which is expressed in rod outer segments but not in eight other tissues (14), SCALD is expressed in a wide variety of organs, including testis, liver, and retina. Considered together, these findings suggest that SCALD plays a role in the metabolism of short-chain aldehydes in addition to retinaldehydes, and therefore we propose the less restrictive name SCALD.

EXPERIMENTAL PROCEDURES

Materials—We obtained 4-hydroxy-trans-2-nonenal from Cayman Chemical; other aldehydes from Aldrich; steroids from Steraloids; ponasterone A from Invitrogen; Zvitert gent-3-16 detergent from Calbiochem; CAPS detergent from Novagen; d-[3H]glucose (20 Ci/mmol) from American Radiolabeled Chemicals, Inc.; mouse monoclonal anti-His IgG from Qiagen; and other chemicals and mouse monoclonal anti-Flag IgG from Sigma. The prRDH-His expression plasmid (14) was kindly provided by Jeremey Nathans (Johns Hopkins University School of Medicine).

General Methods—Lipoprotein-deficient newborn calf serum was prepared as described (15). Northern blot hybridization of total RNA was carried out as described (9). Filters were exposed to X-Omat blue film (Kodak) with intensifying screens at C for 16 h. Quantitative real-time PCR was carried out as described (16), using SCALD gene-specific primers (5′-GGCCAGCTCTGCCGCTCTTGCTCTCC-3′) provided by the manufacturer and the 3′-primer based on the 5′-sequence of the SCALD fragment (5′-GTCCGACACTGAGGGCTCTTCTC-3′). The 3′-RACE fragment was amplified with the 5′-primer based on the 3′-sequence of the SCALD fragment (5′-GGAGAGGAAGCCTCAAGTGGCTGAG-3′) and the 3′-primer API. 5′ and 3′ RACE fragments were sequenced, and the full-length SCALD cDNA was amplified from the cDNA library using primers 5′-GATCGTCTTCCAGAGGCTCAGAACCCTAATAGGGCTCC-3′ and 5′-GTCAGCCCGAGGAAATGGCAAGGCGCACAGGT-3′ based on 5′- and 3′-RACE fragment sequences, respectively. The 2.7-kb PCR product was subcloned into the pT-Adv TA cloning vector (Clontech). The resulting plasmid was designated pT-Adv-SCALD and sequenced.

Expression Plasmids—pSCALD-Flag and pSCALD-His encode epitope-tagged versions of mouse SCALD and were generated by PCR with pT-Adv-SCALD as a template with the 5′-primer (5′-CTGAGATGTTTGCGATGATGATGCCAATCCACTGGGAG-3′) and the 3′-primer (5′-TTAATTGATGATGATGATGATGATGCCAATCCACTGGGAGGCCCAGCAGGT-3′) for the FLAG-tagged version and the 3′-primer based on the 3′-sequence of SCALD for the His-tagged version. The PCR product was cloned into the pTarget vector (Promega) by TA cloning. A similar construct without a COOH-terminal tag was generated by PCR and designated pSCALD. pHis-SCALD encodes a histidine-tagged version of SCALD and was generated by PCR using the 5′-primer (5′-GTAATGAGATGATGATGATGATGCCAATCCACTGGGAGGCCCAGCAGGT-3′) and the 3′-primer (5′-TTAATTGATGATGATGATGATGATGCCAATCCACTGGGAGGCCCAGCAGGT-3′) for the FLAG-tagged version and the 3′-primer based on the 3′-sequence of SCALD for the His-tagged version. The PCR product was cloned into the pTarget vector (Promega) by TA cloning. A similar construct without a COOH-terminal tag was generated by PCR and designated pSCALD. pHis-SCALD encodes a histidine-tagged version of SCALD and was generated by PCR using the 5′-primer (5′-GTAATGAGATGATGATGATGATGCCAATCCACTGGGAGGCCCAGCAGGT-3′) and the 3′-primer based on the 3′-sequence of SCALD for the His-tagged version. The PCR product was cloned into the pTarget vector (Promega) by TA cloning. A similar construct without a COOH-terminal tag was generated by PCR and designated pSCALD. pHis-SCALD encodes a histidine-tagged version of SCALD and was generated by PCR using the 5′-primer (5′-GTAATGAGATGATGATGATGATGCCAATCCACTGGGAGGCCCAGCAGGT-3′) and the 3′-primer based on the 3′-sequence of SCALD for the His-tagged version. The PCR product was cloned into the pTarget vector (Promega) by TA cloning. A similar construct without a COOH-terminal tag was generated by PCR and designated pSCALD.
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**Fig. 1.** Amino acid sequence and membrane localization of SCALD. A, alignment of human and mouse sequence. Residue numbers are shown on the right. Identical residues are boxed. Overbars denote the two signature sequences for the superfamily of short-chain dehydrogenases/reductases (37). GenBank accession numbers for mouse and human SCALD are AF474027 and AAF89632, respectively. Human SCALD is also called PSDR1 (10), RDH11 (12), and RalRI (11). B, Kyte-Doolittle hydrophathy plot of mouse SCALD. The residue-specific hydrophathy index (38) was calculated over a window of 18 residues using DNA STAR software Version 5.0, C, membrane localization of SCALD by immunoblot analysis of cell fractions. On day 0, cultured HEK-293 cells were set up and transfected on day 1 with either empty vector (Mock) or pSCALD-Flag as described under "Experimental Procedures." On day 2, the cells were harvested, and whole cell (Whole), supernatant cytosolic (S), and pelleted membrane (P) fractions were isolated (lanes 1–4). Aliquots of the membrane fraction were treated with Na2CO3 or Nonidet P-40 as described under "Experimental Procedures" and fractionated again into supernatant (S) and pellet (P) fractions (lanes 5–8). Aliquots of protein (30 μg) from the different fractions were subjected to SDS-PAGE and immunoblotted with 5 μg/ml of mouse monoclonal anti-Flag antibody. Coupled with keyhole limpet hemocyanin. For immunoblotting, whole serum was used at a 1:1000 dilution. For immunohistochemistry, the antibody was affinity-purified on a column in which the above synthetic peptide was coupled to seagrose beads using a SulfoLink kit (Pierce). Cell Culture, Transfection, and Fractionation—On day 0, human embryonic kidney 293 (HEK-293) cells were plated at a density of 2 × 10^6 cells/100-mm dish in medium A (Dulbecco's modified Eagle's medium containing 100 units/ml penicillin and 100 μg/ml streptomycin sulfate) supplemented with 10% (v/v) fetal calf serum. On day 1, cells were cotransfected with 0.5 μg of pVA1 (18) plus 2 μg of one of the following vectors: pTarget, pSCALD, pSCALD-Flag, pSCALD-His, or prRDH-His using the MBS transfection kit (Stratagene) according to the manufacturer's instructions. Briefly, the cells were switched to medium A supplemented with 6% (v/v) MBS solution. DNA suspensions (2.5 μg/dish) were incubated with the cells for 3 h at 35 °C under 3% CO2. Monolayers were then switched back to medium A supplemented with 10% fetal calf serum. Cells were harvested on day 2 for preparation of cell fractions. M19 cells are a mutant line of CHO-K1 cells that lack nuclear SREBP1a and in the absence or presence of 1 μM ponasterone A for N-BP1a cells and in the absence or presence of 10 μM ponasterone A for N-BP2 cells (20). Cells were harvested on day 3 for preparation of membrane fractions. Murine embryonic fibroblasts (MEF-1 cells) were plated at a density of 5 × 10^5/100-mm dish in medium A supplemented with 10% fetal calf serum. On day 1, the monolayers were switched to medium A supplemented with 10% lipoprotein-deficient serum containing 0.2% ethanol in the absence or presence of sterols (10 μM cholesterol and 1 μM 25-hydroxycholesterol), 50 μM compactin, and 50 μM sodium mevalonate as indicated. Cells were harvested on day 2 for preparation of membrane fractions. For SDS-PAGE, cell fractionation was carried out as described (21), and the membrane fractions were either resuspended in SDS buffer and subjected to electrophoresis or treated with 100 mM Na2CO3 for 15 min at room temperature or 0.25% Nonidet P-40 for 30 min at 4 °C, pelleted at 10^5 × g to separate soluble and insoluble material, and then subjected to electrophoresis (21). For assays of enzymatic activity, a microsomal fraction (10^5 × g pellet) was prepared as described (22). Animal Tissues—Tissues were collected immediately after sacrifice of the animals. For Northern blots, tissues were quickly frozen in liquid nitrogen for subsequent extraction of RNA. For immunoblotting, all operations were done at 4 °C. Tissues were homogenized with a Polytron into ~5 volumes of sucrose buffer (5 mM Hepes at pH 7.6, 250 mM sucrose, 0.3% (v/v) Triton X-100, and a mixture of protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leu-
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A. Northern Blot of Mouse Tissues

| Tissue | Genotype | WT | 1c | WT | 1c | WT | 1a | 2 |
|--------|----------|----|----|----|----|----|----|---|
|        |          |    |    |    |    |    |    |   |
| SCALD  |          |    |    |    |    |    |    |   |
| β-actin|          | 1.4| 2.4| 1.4| 2.4| 1.4| 2.4|   |

B. Quantitative PCR of SCALD in Liver

| SCALD mRNA Level (arbitrary units) | WT | SCAP<sup>−/−</sup> |
|-----------------------------------|----|----------------|
| Control                           | 1.0| 1.0           |
| F                                 | 0.5| 0.5           |
| RF                                | 0.5| 0.5           |

C. Immunoblot of N-BP Cells

| Cell Line | Sterols | Compactin |
|-----------|---------|-----------|
| N-BP1a    | -       | -         |
| N-BP2     | +       | +         |

D. Immunoblot of MEF-1 Cells

| Sterols | Compactin |
|---------|-----------|
| -       | +         |
| +       | +         |

peptin, and 10 μg/ml pepstatin A<sup>1</sup>) and clarified by centrifugation at 10<sup>3</sup> × g for 10 min. For histology, tissues were harvested from anesthetized mice and fixed via transcardial perfusion with 4% (w/v) formaldehyde freshly prepared from paraformaldehyde (in situ hybridization) or Bouin’s fixative (immunohistochemistry). Subsequent paraffin processing, embedding, and sectioning were performed by standard procedures (23, 24).

RNA in S itu Hybridization—Probe template corresponding to SCALD cDNA nucleotides 3 to 413 (where +1 corresponds to A of initiator methionine) was amplified by PCR using primers (5<sup>′</sup>-GTTCGGATTCTCGTCTCCTCTGC-3<sup>′</sup> and 5<sup>′</sup>-CGTCTGAGTCTTCAGTAGTAGG-3<sup>′</sup>) and cloned into pCR2-TOP (Invitrogen). 32P-Labeled sense and antisense probes were generated by Sp6 and T7 RNA polymerases, respectively, from linearized cDNA templates by in vitro transcription using the Maxiscript kit (Ambion, Inc., Austin, TX). Radioisotopic in situ hybridization was performed as described (25). Autoradiographic exposure ranged from 21 to 28 days at 4 °C.

Immunohistochemistry—Serial sections of testis and retina were incubated with either 0.8 μg/ml of affinity-purified anti-SCALD antibody or 10 μg/ml of preimmune serum according to immunoperoxidase methods described previously (26, 27) for testis and immunofluorescence methods (28) for retina.

Assay of Enzyme Activity—All reactions were carried out in buffer D (100 mM potassium phosphate at pH 7.2, 200 mM NaCl, 1 mM dithiothreitol, 2 mg/ml bovine serum albumin, 1% glycerol, and 150 μM NADPH). Reactions with detergent-solubilized recombinant SCALD were carried out in a final volume of 0.5 ml of buffer D containing either 200 μg of microsomal protein (for reactions with steroids and carbonyl compounds) or 10 μg of microsomal protein (for reactions with retinaldehydes). All reactions were initiated by a 1-μl addition of ethanol containing the indicated concentration of substrate.
FIG. 3. Reduction of nonanal by detergent-solubilized recombinant SCALD. Enzyme reactions were monitored spectrophotometrically for 5 min at room temperature as described under “Experimental Procedures.” A, enzyme concentration curve. Increasing amounts of detergent-solubilized SCALD were added to the standard reaction at pH 7.5 in the presence of 100 µM nonanal. B, pH curve. Each reaction was carried out at the indicated pH in the presence of 1 µg of SCALD and 100 µM nonanal. C, NADPH concentration curve. The same conditions as in B were used with the indicated concentration of NADPH at pH 7.2. D, nonanal concentration curve. The same conditions as in B were used with the indicated concentration of nonanal at pH 7.2. B-D, a blank value (~0.4 nmol/min/tube) based on the rate of disappearance of NADPH in parallel reactions lacking SCALD was subtracted from each measured value. Insert in A, SDS-PAGE (12% gel) of detergent-solubilized SCALD (2 µg), followed by staining with GelCode Blue reagent (Pierce).

With steroids and carbonyl compounds as substrates, SCALD activity in detergent-solubilized recombinant protein was measured by monitoring the rate of NADPH oxidation spectrophotometrically at 340 nm during a 5-min incubation at room temperature (~22 °C) using an Ultrospec 3000 spectrophotometer (Pharmacia Biotech). Enzyme activity was calculated based on an absorption coefficient of 6220 M⁻¹ cm⁻¹ at 340 nm for NADPH.

With steroids and carbonyl compounds as substrates, SCALD and prRDH activity in microsomes from transfected HEK-293 cells was measured by monitoring the production of 3H-labeled products. For these experiments, (4S)-[4-3H]NADPH was prepared from D-[1-3H]glucose. Isolated microsomes were solubilized using Nonidet P-40. The rate of NADPH oxidation was 85.4% identical to the human PSDR1 (10), also named RalRI (11) and RDH11 (12); it is 100% identical to the sequence reported by Moore et al. (30). SCALD belongs to the family of short-chain dehydrogenase/reductases (31, 32). Members of this family are NAD(P)(H)-dependent oxidoreductases. The various members exhibit only 15–30% identity, but all possess two highly conserved motifs that are the signature of this family. These motifs are overlined in Fig. 1A. The first motif, GXXGXXG, is in the coenzyme-binding domain, and the second motif XXXXX is involved in catalytic activity. The hydropathy plot of SCALD (Fig. 1B) shows a protein of 316 amino acids with a hydrophobic NH₂-terminal segment of 20 amino acids that likely functions as a signal sequence for insertion into the endoplasmic reticulum. If this sequence is not cleaved, it could also serve as a membrane anchor. As shown in Fig. 1C, when a FLAG-tagged version of SCALD was expressed in HEK-293 cells through transfection, the protein was bound to membranes and could not be dissociated by treatment with sodium carbonate, but it was solubilized with Nonidet P-40. This indicates that SCALD remains bound to the membrane through its uncleaved hydrophobic NH₂-terminal sequence. This finding is in agreement with that of Kedishvili et al. (11).

Fig. 2A shows that SCALD was overexpressed in white and...
brown adipose tissue of the aP2-SREBP-1c mice as determined by Northern blotting of the mRNA (lanes 1–4). The SCALD mRNA was also increased in livers of previously described transgenic mice that overexpress the nuclear forms of SREBP-1a or SREBP-2 in liver (lanes 5–7). In livers of wild-type mice, the amounts of nSREBPs are known to decline with fasting and rise with the refeeding of a low-fat diet (33). Fig. 2B shows that the amount of SCALD mRNA declined with fasting and rose with refeeding, consistent with regulation by nSREBPs. This conclusion was supported by the finding that SCALD mRNA is markedly reduced in livers of targeted mice with a disruption in the hepatic gene encoding SREBP-2 (Fig. 2C). In these mice, the SCALD mRNA declined only slightly with fasting, and it failed to show the normal rise with refeeding, again supporting the conclusion that hepatic SCALD is regulated by SREBPs.

To test this regulation in another way, we used N-BP cells (20), which are lines of Chinese hamster ovary cells that have been engineered to express individual isoforms of nSREBPs in response to induction by the steroid ponasterone (Fig. 2C). We blotted these cells with an antibody directed against a peptide derived from SCALD (see “Experimental Procedures”). Immunodetectable SCALD rose when ponasterone induced the production of nSREBP-1a (lane 2) or nSREBP-2 (lane 4). The SCALD protein was induced in mouse embryonic fibroblasts (MEF-1 cells) that were treated with the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor compactin (Fig. 2D, lane 3), and it was reduced when sterols were added (lane 2). All of these findings are consistent with regulation of SCALD by SREBPs.

Fig. 4. Substrate specificity of SCALD: comparative activity of detergent-solubilized enzyme (A) and microsomal enzyme (B). Assays in A were carried out with 1 μg of detergent-solubilized recombinant SCALD using the spectrophotometric assay as described under “Experimental Procedures.” Assays in B were carried out with 200 μg of microsomal protein from pSCAP-transfected HEK-293 cells using the [3H]NADPH assay as described under “Experimental Procedures.” In both assays, the indicated substrates were added at a final concentration of 100 μM. The results (means of triplicate assays) are expressed as relative activities compared with the activity of nonanal, which was set at a value of 1.0. The activity for nonanal in A and B were 4.5 nmol/min/tube and 9821 cpm/h/tube, respectively.
for the assay with purified SCALD. In general, we observed a close correlation between the activities of the purified and microsomal enzyme preparations (Fig. 4), although there were some exceptions. Neither enzyme preparation showed significant reductive activity toward the keto-containing steroids progesterone and dehydroisoandrosterone, whereas both preparations were active against a variety of short- and medium-chain aldehydes of 8–10 carbons containing zero to two double bonds.

We could not use the spectrophotometric assay to measure reduction of retinaldehydes because these substrates absorb light at 340 nm. However, we could use the [3H]NADPH reduction assay to measure the reduction of retinaldehydes in the SCALD-expressing microsomes. At the standard substrate concentration of 100 μM, the reduction of all-trans-retinal and 9-cis-retinal was markedly slower than the reduction of nonanal (Fig. 4B).

To measure retinaldehyde reduction with the purified recombinant enzyme, we use HPLC to separate the retinol products from the retinaldehyde substrates (Fig. 5). For comparative purposes in the same experiment, we measured the reduction of all-trans-retinal and 9-cis-retinal using the spectrophotometric assay. The results showed that the $K_m$ values for all-trans-retinal (Fig. 5B) and 9-cis-retinal (Fig. 5C) were in the same range as the $K_m$ for nonanal (Fig. 5A). The $V_{max}$ for the retinaldehydes was lower than the $V_{max}$ for nonanal.

To test the significance of the broad substrate specificity of SCALD, we compared the activity of this enzyme with the activity of prRDH, an enzyme that is specifically expressed in retinal rod and cone outer segments and that appears to be specialized for the reduction of all-trans-retinal (14). We obtained an expression plasmid encoding prRDH as a generous
gift from the laboratory of Jeremy Nathans. HEK-293 cells were transfected with a cDNA encoding His-tagged prRDH or a cDNA encoding His-tagged SCALD. Microsomes were subjected to immunoblotting with an anti-His antibody, and the expression levels of the two proteins were comparable (Fig. 6A).

The microsomes were assayed for their ability to reduce nonanal using the [3H]NADPH assay and their ability to reduce all-trans-retinal using the HPLC assay. The apparent \( K_m \) for the SCALD-mediated reduction of all-trans-retinal was much lower with the microsomal preparation (1.3 \( \mu \)M) than it was for the purified enzyme in detergents (57 \( \mu \)M, see Fig. 5). This difference was reproducible with several enzyme preparations. The microsomal preparation of prRDH had a similarly low \( K_m \) for all-trans-retinal (Fig. 6C). However, whereas SCALD was active on nonanal, prRDH showed no measurable activity (Fig. 6B).

Human PSDR1 was reported to have greatest expression in the prostate (10), whereas mouse PSDR1, which is identical to mouse SCALD, was reported to be expressed most highly in testis and liver and much less in prostate (30). The Northern blots of Fig. 7A confirm the highest levels of SCALD expression in testis with relatively high levels in liver, adrenal, and ovary. Expression of SCALD was barely detectable in mouse prostate. A similar relative distribution of SCALD (testis > liver >> prostate) was seen in rat tissues (Fig. 7B). Immunoblots of mouse tissues with the anti-SCALD antibody revealed similar levels of protein expression in liver and testis despite the differences in mRNA levels. The apparent molecular mass of the protein was 31 kDa, which is consistent with the size predicted from the mRNA sequence. This antibody was not potent enough to detect SCALD protein in any of the other mouse tissues that were examined (Fig. 7C).

In situ hybridization experiments revealed that SCALD expression in testis was confined to the seminiferous tubules (Fig. 8A). Only a narrow layer of the tubules was visualized, suggesting that SCALD is expressed in a particular stage of sperm cell development. No hybridization signal was obtained with the control sense probe (Fig. 8B). Higher magnification suggested that SCALD mRNA is concentrated in the peripheral layer of the seminiferous tubule (Fig. 8C), which contains pachytene spermatocytes as indicated by bright-field examination of the same hematoxylin-stained section (Fig. 8D). Immunohistochemical staining confirmed the expression of SCALD protein in pachytene spermatocytes (Fig. 8, E and F). The protein was concentrated in a perinuclear body consistent with localization to the Golgi complex. Remarkably, we were unable to detect SCALD mRNA or protein in either less mature or more mature sperm, suggesting that the expression of SCALD is highly stage-specific in sperm differentiation.

Immunohistochemical staining of the retina with an affinity-purified anti-SCALD antibody revealed specific immunoreactivity within distinct layers of the retina (Fig. 9, top). Punctate cytoplasmic staining was evident in the outer segment of the layer of rods and cones. In the outer plexiform layer, the stain-
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The data in this paper extend our knowledge of the mammalian short-chain dehydrogenase/reductase that is known variously as PSDR1, RalR1, RDH11, and SCALD. For purposes of this discussion, we will use the name SCALD. The new findings are: 1) transcription of the SCALD gene is regulated by SREBPs. As a result, in tissue culture cells the gene is transcribed actively when the cells are deprived of sterols and is repressed when sterols are abundant. In liver, SCALD mRNA declines with fasting and reappears with refeeding; 2) in contrast to the reported enrichment of the corresponding human enzyme in prostate, SCALD is expressed at very low levels, if at all, in the prostate of mice and rats, and it is enriched at the mRNA level in testis, liver, adrenal, and ovary of rodents. At the protein level, the enzyme is present at equal levels in mouse testis and liver and at much lower levels in other tissues. Immunohistochemical studies of the mouse retina showed that SCALD is expressed in four layers of the retina in contrast to the more restricted expression of RDH11 (exclusive ly in bovine and human retinal pigment epithelium) (12) and prRDH (exclusively in the outer segments of macaque rods and cones) (14); 3) in mouse testis, SCALD shows a highly restricted pattern of expression in pachytene spermatocytes, but not in progenitor spermatogonia or in mature sperm; 4) in contrast to the previously described enzyme designated prRDH, which is expressed in retinal rods and reduces all-trans-retinal, purified recombinant SCALD shows a broad substrate specificity, reducing short-chain aliphatic aldehydes such as nonanal as actively as it reduces retinals. Considered together, these data indicate that mouse SCALD and its human ortholog (PSDR1/RalR1/RDH11) have functions that may be much broader than simply reducing retinaldehydes.

The reason for the tight regulation of SCALD transcription by SREBPs is unknown, but the following hypothesis must be considered. SREBPs are known to enhance the synthesis of fatty acids, their elongation to stearate, and their subsequent unsaturation to yield oleate (1). In addition, through their induction of the low density lipoprotein receptor mRNA, SREBPs enhance the cellular uptake of lipoproteins that contain polyunsaturated fatty acids, especially linoleate and arachidonate. Unsaturated fatty acids are subject to oxidation by molecular oxygen (13). Oxidation yields a wide variety of C2–12 fatty aldehydes, including 4-hydroxy-2-nonenal and nonanal. Such aldehydes are toxic to cells because they form Schiff bases with lysine residues in cellular proteins. Hence, as a protective maneuver SREBPs may induce SCALD so as to reduce these toxic aldehydes to nontoxic alcohols. If this hypothesis is correct, it would predict that fatty aldehydes or their protein adducts should accumulate when SREBP activity is stimulated in livers of knock-out mice that lack the SCALD gene. We are currently in the process of preparing and studying SCALD knock-out mice for this purpose.

The reason for the expression of SCALD specifically in pachytene spermatocytes remains to be elucidated. Recently, Wang et al. (35) reported the specific expression in mouse and rat spermatogenic cells of an aberrantly spliced mRNA that encodes a truncated form of SREBP-2 that terminates prior to the membrane-spanning sequence. We have shown previously that such a truncated SREBP-2 (encoded by a mutant gene in sterol-resistant Chinese hamster ovary cells) goes directly to the nucleus and activates transcription without a requirement for proteolytic processing, and hence it is not down-regulated in the nucleus and activates transcription without a requirement for proteolytic processing, and hence it is not down-regulated.
by sterols (36). Further studies are necessary to determine whether the specific expression of SCALD in pachytene spermatocytes is triggered by the endogenous truncated form of SREBP-2.

Acknowledgments—We thank Jeremy Nathans for providing the pDRDH expression vector, Richard Gibson for excellent help with animals, Scott Clark for invaluable technical assistance, Linda Donnelly and Lisa Beatty for excellent help with tissue culture, and Jeff Cormier for DNA sequencing.

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Characterization of Mouse Short-chain Aldehyde Reductase (SCALD), an Enzyme Regulated by Sterol Regulatory Element-binding Proteins
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J. Biol. Chem. 2003, 278:32380-32389.
doi: 10.1074/jbc.M304969200 originally published online June 13, 2003

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