Multiple retinoic acid responsive cDNAs were isolated from a high density cDNA microarray membrane, which was developed from a cDNA library of human tracheobronchial epithelial cells. Five selected cDNA clones encoded the sequence of the same novel gene. The predicted open reading frame of the novel gene encoded a protein of 319 amino acids. The deduced amino acid sequence contains four motifs that are conserved in the short-chain alcohol dehydrogenase/reductase (SDR) family of proteins. The novel gene shows the greatest homology to a group of dehydrogenases that can oxidize retinol (retinol dehydrogenases). The mRNA of the novel gene was found in trachea, colon, tongue, and esophagus. In situ hybridization of airway tissue sections demonstrated epithelial cell-specific gene expression, especially in the ciliated cell type. Both all-trans-retinoic acid and 9-cis-retinoic acid were able to elevate the expression of the novel gene in primary human tracheobronchial epithelial cells in vitro. This elevation coincided with an enhanced retinol metabolism in these cultures. COS cells transfected with an expression construct of the novel gene were also elevated in the metabolism of retinol. The results suggested that the novel gene represents a new member of the SDR family that may play a critical role in retinol metabolism in airway epithelia as well as in other epithelia of colon, tongue, and esophagus.

Vitamin A (retinol) and its metabolites (retinoids) are essential to the development and maintenance of the airway epithelial phenotype (1–4). Epithelial tissues, including the airway epithelium, are vitamin A target tissues that require retinoids (5–7). Vitamin A metabolites, such as all-trans-retinoic acid (RA)\(^1\) and 9-cis-RRA, are important regulators for gene transcription as the ligands for various transcriptional factors of the retinoic acid receptor (RAR) and retinoid X receptor (RXR) families (for review, see Ref. 8). Extensive progress has been made in determining the specificity of these RA metabolites with the interactions with these RARs and RXRs (8, 9). As compared with these RA metabolites, retinol is not as potent as those RAs in terms of the interactions with these receptors and in terms of transcriptional regulation (10, 11). Thus, it was suggested that retinol has to be metabolized to various RAs to exert its biological activity (11). However, the mechanism by which vitamin A and its metabolites exert this activity to regulate airway development and the maintenance of mucociliary functions in epithelium is unknown.

As lipid soluble compounds, retinoids can readily cross the membrane into any cell in the body from the plasma circulation. The major form of retinoid in plasma circulation is all-trans-retinol at about 1.5–2.0 \(\mu\)g (12, 13). However, the level of RA in the plasma is around 4–14 nM (14, 15), which may be insufficient to supply the needed cellular RA level for its biological activity in vitamin A target tissues and cells. In addition, in some vitamin A target tissues and cells, such as skin and keratinocytes (16), the access to blood vessels can be quite limited. Thus, there is a need in these vitamin A target cells for an efficient machinery to transport and to metabolize plasma retinol into various RA metabolites.

The production of RA from retinol can take place within the cell if the cell contains enzymes that can sequentially oxidize retinol to retinaldehyde and retinaldehyde to RA. The reversible oxidation of retinol to retinaldehyde has been suggested to be the rate-limiting step in the metabolism of retinol to retinoic acid and therefore the step most likely to be tightly regulated by the cell (17, 18). The second step, the oxidation of retinaldehyde to RA, seems to be irreversible (reviewed in Ref. 19).

Consistent with the first step in this model of retinol metabolism in vitamin A target cells, there have been reports of multiple enzymes with retinol dehydrogenase activities that are isofrom-specific or cell type-specific. These enzymes include (but are not limited to) RODH-I (17), RODH-II (20), RODH-III

\(^{1}\) The abbreviations used are: RA, retinoic acid; RAR, retinoic acid receptor; RXR, retinoid X receptor; RODH or RoDH or RODH, retinol dehydrogenase; TBE (human) tracheobronchial epithelia; SDR, short chain (alcohol) dehydrogenase/reductase; hRDH-TBE, TBE, cell-specific human retinol dehydrogenase; FCR, polymerase chain reaction; UTR, untranslated region; HPLC, high performance liquid chromatography; bp, base pair(s).
(21), RDH-4 (22), hRDH-E (16), 9- and 11-cRDH (23–25), RDH-5 (26–29), RDH-6 (30), and RDH-7 (31). All of these enzymes are members of the short chain (alcohol) dehydrogenase/reductase (SDR) gene superfamily.

Enzymes in the alcohol dehydrogenase and cytochrome P450 families have been implicated in the oxidation of retinaldehyde to retinoic acid, which is the second step in the metabolism of retinol to retinoic acid. There are reports of retinaldehyde-specific dehydrogenases that catalyze this step (32–38). Of particular interest, at least one of these retinaldehyde dehydrogenases is specifically expressed in tracheal epithelial cells in rat, with a putative role in the conversion of retinaldehyde to retinoic acid in these cells. However, the critical enzyme that converts retinol to retinaldehyde is still unknown in airway epithelial cells.

In this communication, we report the cloning and characterization of a novel airway epithelial cell-specific SDR gene from a cDNA library derived from primary human tracheobronchial epithelial (TBE) cells, using a differential hybridization approach on a high density cDNA microarray membrane. Five independently selected RA responsive cDNA clones encoded the same novel gene. The conceptual translation of the novel cDNA sequence encodes a protein that contains amino acid motifs that are conserved in the SDR family of genes. Within the SDR superfamily, the novel gene is most homologous to the retinol dehydrogenases.
this novel gene represents a new member of the SDR family that may play a critical role in retinoid metabolism in airway epithelia.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and RNA Isolation**—Human airway tissues were obtained from the University of California at Davis Medical Center with donor consent. The protocol for obtaining human tissues has been approved and periodically reviewed by the campus Human Subject Review Committee. TBE cells were isolated from these tissues by a protease dissociation procedure and cultured in a serum-free hormone-supplemented medium as previously described (39). Briefly, cells were grown in 6-well tissue culture plates either without additional substratum (TC) or with collagen gel substratum (Cg) in an air-liquid biphasic culture insert chambers (Transwell™ chamber, Corning/Costar 3450, Corning, NY) without (Bi) or with collagen gel substratum (Bi-Cg). The serum-free hormone-supplemented medium was slightly modified from that described in Ref. 39; the modified medium contained the supplements insulin (5 μg/ml), transferrin (5 μg/ml), EGF (10 ng/ml), cholera toxin (20 ng/ml), dexamethasone (0.1 μg), bovine hypothalamus extract (15 μg/ml), bovine serum albumin (1 mg/ml), and all-trans-RA (30 nM, Fluka, Milwaukee, WI) in a nutrient medium containing 1:1 of Ham's F12/Dulbecco's modified Eagle's medium. This modified culture medium allows epithelial cells to better grow and express mucociliary differentiation than before in culture, especially under the Bi-Cg condition. For both Bi and Bi-Cg culture conditions, cells were immersed in the culture medium for 7 days and then the Transwell™ chambers were lifted up in between the air and liquid interface for the remaining days in culture. For dose response and time course experiments, cultures were maintained for 7 to 10 days without all-trans-RA supplementation and then RA at 30 nM or at various concentrations (1–1000 nM) was added accordingly as described in the experiments. After various lengths of time in culture, total RNA was isolated from cultured cells by a single step acid guanidium thiocyanate extraction method (40).

**Cloning and Sequencing of the Novel cDNA**—Thirty thousand cDNA clones were derived from primary human TBE cells that had been cultured for more than 30 days under an air-liquid interface culture condition in the completed medium containing both the hormonal supplements and all-trans-RA. Under such an in vitro culture system, the human TBE cells differentiated into a muccociliary epithelium resembling that seen in vivo. The cDNA clones were packaged using a pBK CMV plasmid vector (Stratagene, La Jolla, CA).

From the 30,000 cDNA clones, we developed a high density microarray membrane was hybridized simultaneously with two-color cDNA probes, magenta- and cyan-based, derived from all-trans-RA-treated anhuman TBE cells, respectively, as described before (41). Based on a quantitative ratio of cyan/magenta either greater than 5 or less than 0.2, clones were selected for further characterization. These characterizations included the use of Northern blot hybridization to further confirm the differentially expressed nature of the corresponding messages in these clones, DNA sequencing to allow searches for homologous genes of known function to provide clues as to the function of a novel gene, and, in some cases, in situ hybridization to elucidate cell type-specific gene expression.

DNA sequencing was carried out at the DBS Automated DNA Sequencing Facility, UC-Davis. DNA sequence data was analyzed with Lasergene software (DNASTAR Inc., Madison, WI) and with the online GCG Software package SeqWeb (Madison, WI). Sequence homology to published sequences in public databases was determined by the BLASTN or BLASTp program at the National Center for Biotechnology Information (NCBI) through Internet services. The phylogenetic tree was compiled by the GCG program GrowTree with the following parameters: Kimura distance correction method, UPGMA tree construction method, scoring matrix blossum 62, 8-residue-gap creation penalty, 2-residue-gap extension penalty, 1000-residue maximum sequence input range, 6000-residue maximum gap character insertion allowed, with all branches of the same length (a cladogram).

**Plasmids**—All five cDNAs that represent the novel gene are in the vector pBK-CMV (Stratagene) with the 5'-end at the T3 side of the multiple cloning site. PCR using any of these cDNAs as template, with insert-specific primers (forward primer, 5'-TGAGCAAGTCCACACAAGTCATTG3'; reverse primer, 5'-GCAGTAAATGATGCTTCTAG3') generated a fragment of the cDNA containing mostly coding sequence; this PCR product was used to probe Northern blots.

For FLAG tagging, the entire coding region of the novel cDNA was fused in frame 3' to a FLAG tag in the vector pFLAG CMV2 (Sigma, St. Louis, MO). This was done by introducing a unique EcoRI site just upstream of the presumed translational start codon (see Fig. 1C). The FLAG tag was introduced using PCR; the forward primer (5'-GGGGAATTCAATGCTCTTTTGGGTGCTAG) altered five bases of the novel gene's 5'-UTR (the underlined bases), whereas the reverse primer was a commercially available T7 primer. The resulting amplicon was digested with EcoRI and KpnI and inserted into pFLAG-CMV2, which had been cut with the same enzymes. The vector/insert junction was verified by nucleotide sequencing.

**Northern Analysis and 5'-Primer Extension**—Twenty micrograms of total RNA were fractionated on a 0.6% agarose gel and transferred overnight to a Nytran N+ nylon membrane. RNA was fixed to the membrane by UV cross-linking (Stratallinker, Stratagene). The PCR product derived from the novel cDNA was labeled with [α-32P]dCTP (ICN, Costa Mesa, CA) to a specific activity of ~2 × 10^7 dpm/μl with a Ready-To-Go random primer labeling kit (Amersham Pharmacia Biotech Life Sciences, Arlington Heights, IL). The membranes were prehybridized in hybridization solution (6× SSC, 0.5% SDS, 0.01 μm EDTA, 0.5% disodium pyrophosphate, 5× Denhardt's solution) at 68 °C for a minimum of 4 h followed by hybridization in the same solution plus specific probe at 68 °C for 16–20 h (overnight). Hybridized membranes were washed once with 2× SSC, 0.1% SDS for 10 min at room temperature and twice with 1× SSC, 0.1% SDS for 30 min at 68 °C. Following the second wash, membranes were checked for excessive radioactivity, and, if necessary, washed in 0.1× SSC and 0.1% SDS for various times at 68 °C. PhosphoImager screens (Molecular Dynamics, Amersham Pharmacia Biotech Life Sciences) and/or X-Omat film (Kodak, Rochester, NY) were exposed to hybridized membranes for various times.

To study the novel gene's expression patterns in normal tissues, Northern blots containing 20 μg of total RNA from various adult primary tissues were prepared, and Northern analysis was carried out as described above. Monkey tissues were obtained from necropsies done at the California Regional Primate Research Center of UC Davis.

The sequence of the full-length cDNA was determined by 5'-extension on RNA from human TBE cultured under the Bi-Cg condition with
30 nm all-trans-RA, using an endlabeled antisense primer (5'-AG-GAGGCTAGGCTGATAGAG-3') and the dideoxynucleotide chain termination method according to the manufacturer's recommendations (Promega, Madison, WI). A genomic PCR chromosome walking fragment from the promoter region of the gene was sequenced in parallel with the 5' extension product using the same endlabeled primer and the Taqtrack™ sequencing kit (Promega).

**In Situ Hybridization**—Clones obtained from the original phage library screening were converted to phagemids according to the manufacturer's protocol (Stratagene). The recombinant plasmids were linearized with EcoRI or XhoI to generate antisense and sense templates, respectively. The linearized templates were transcribed in vitro with T7 and T3 RNA polymerases using MAXIscript™ according to the manufacturer's recommendations (Ambion Inc., Austin, TX) to produce 32P-UTP-labeled antisense and sense probes, respectively. In situ hybridization was carried out as described previously (42).

**Polyclonal Antibody Production and Western Blot Analysis**—A 15-mer oligopeptide antigen was synthesized (Research Genetics, Inc., Huntsville, AL) using deduced amino acid sequence 244–258 of hRDH-TBE (see Fig. 1C). The peptide was conjugated to multiple antigen peptide to increase its antigenicity, and rabbit-based polyclonal antibodies were generated as described before (1). The specificity of the polyclonal antisera was determined by ELISA and Western blot analysis.

For Western blot analysis, cultured cells were harvested as described (43–45). Supernatant protein concentrations were determined by the method of Lowry using the Bio-Rad DC assay (Bio-Rad, Hercules, CA).

**HPLC Analysis of Metabolites**—Retinoid analysis and quantitation were performed using a reverse phase Nova-Pak C18 (8.0 cm × 10 cm) 4-μm pore size analytical column (Waters Associates, Milford, MA). Reconstituted cell extracts were extracted for retinoids under isocratic conditions using an elution solvent mobile phase of methanol/tetrahydrofuran-acetonitrile-isopropyl alcohol (TAI)/v/v/v: 67:10:23 (v/v/v). The TAI solution was comprised of tetrahydrofuran/ acetonitrile/2-propanol (3:1:0.02 v/v/v). Retinoids were detected by UV absorbance at 390 nm. Under these conditions, the system has a lower limit of detection for RA of ~1.3 pmol at a signal-to-noise ratio of 2.5; detection limits for retinol are ~3.1 pmol.

Cell-associated retinoids are expressed as pmol per mg protein. The retinoid per sample was calculated from each peak area normalized to the peak area of the internal standard and from the amount of protein. All HPLC analyzed groups are from duplicate samples. The amount of all-trans-RA is an indirect measure of retinol dehydrogenase activity because the immediate product of dehydrogenase activity is retinaldehyde, which is irreversibly further oxidized to RA.

For calibrations and standards, pure retinoids were made in methanol and their concentrations determined by UV absorbance using published maximal absorbance wavelengths and corresponding molar extinction coefficients (47). The elution position of matching retinoid standards was used to identify specific retinoid peaks that were then quantified by computer integration of the areas under the respective peaks. The purity of the retinoids was verified by determining the absorption spectra of isolated peaks. Accuracy of the method and calibration conditions were further verified using authenticated samples containing known quantities of retinoids (National Bureau of Standards).

**RESULTS**

**Novel RDH cDNA Identification**—Based on differential hybridization results, 79 cDNA clones were selected as RA-responsive clones for further study. Northern blot hybridization confirmed that the corresponding messages of 76 clones were differentially regulated by all-trans-RA. Of the remaining three clones, one was a false positive and the other two were unable to be characterized by Northern blot because of extremely low abundance message levels. Among the 76 differentially expressed clones, 14 encoded novel cDNA sequences. Five of the 14 novel clones, DD13, DD18, DD90, DD91, and DD95, had

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Airway Epithelial Cell-specific SDR

Homology to the SDR Family—Searches of the GenBank \textsuperscript{TM} (nr) database using various incomplete fragments of the novel cDNA sequence as query revealed homology at the DNA level to members of the SDR gene family. A BLAST search of the GenBank \textsuperscript{TM} nr database with the completed cDNA sequence revealed an almost complete identity to three clones identified as retinol dehydrogenase isoforms-1 and -2, and retinol dehydrogenase homolog (GenBank \textsuperscript{TM} accession numbers AF240698, AF240697, and AF067174). No information other than the sequences has been provided for these clones. Alignment of the homologous sequences with the DD13/18/90/91/95 clones indicates that the clones were short in sequence at the 5’-end of the novel gene (Fig. 1A). There are gaps in the alignment of two of the homologous clones, which may represent alternative splicing events.

In addition to the close homology to clones AF240698, AF240697, and AF067174, the newly identified cDNA clone also had homology to multiple members of the SDR superfamily. This included various hydroxysteroid dehydrogenases and oxidoreductases as well as the retinoid-associated SDR subgroup (31) of various tissue- and isoform-specific retinol dehydrogenases. A search of the deduced amino acid sequence of the DD13/18/90/91/95 gene revealed the presence of four motifs, which are conserved in 70% of SDR gene family members (48). These motifs are the amino acid residues GXXXGXG at 36–42, YXXX at 176–180, LXNNAG at 109–114, and PG at 206–207 (Fig. 1C, boxed amino acid sequences).

Expression Pattern of hRDH-TBE in Tissues and Cultured Cells—To verify tissue-specific gene expression, RNA samples from several primate tissues were used. Northern blot hybridization demonstrated the presence of hRDH-TBE message in trachea, colon, tongue, and esophagus (Fig. 3). In these tissues, a single hybridized band was observed. This band was similar in size to the 18 S RNA band (1874 nucleotides, Ref. 49) based on comparison to stripped and reprobed Northern blots. The rest of the tissues (heart, kidney, liver, lung, stomach, small intestine, and spleen) had no detectable message.

To determine cell type-specific hRDH-TBE gene expression, in situ hybridization was carried out on sections derived from primate airway and lung tissues. As shown in Fig. 4, only the surface epithelial cells reacted with the antisense probe to the hRDH-TBE gene. The hybridization signal was localized to the ciliated cell type (Fig. 4A). This pattern was persistently seen in all the surface airway epithelium (Fig. 4B), including that of the distal region (data not shown). There was no positive hybridization signal in the alveolar region (data not shown). The control with sense probe showed no positive hybridization (Fig. 4C).

Regulation of hRDH-TBE Gene Expression in Culture by Retinoids—Northern blot analysis demonstrated that hRDH-TBE gene expression in cultured human TBE cells was regulated by all-trans-RA. As shown in Fig. 5A, hRDH-TBE message was elevated in all of the all-trans-RA-treated cultures, regardless of the culture condition. Comparison of the band intensities suggested that the elevation was most significant in the Bi-Cg condition.

Dose-response experiments demonstrated a dose-dependent stimulation of hRDH-TBE message in culture by all-trans-RA and 9-cis-RA (Fig. 5, B and C). Some differences in transcript elevation between the two RA isoforms were observed; for example, 9-cis-RA seemed to elevate hRDH-TBE message at lower doses than all-trans-RA. A time course study demon-
strated that the stimulation by all-trans-RA was an early event that could be seen two hours after treatment (Fig. 5D).

**Immunological Identification of hRDH-TBE Protein in Cultured Cells and Tissue Sections**—A polyclonal antibody specific to the deduced amino acid residues 244–258, EKLKD-KLGKNRSTYN, was developed. The ability of the polyclonal antibody to specifically recognize the hRDH-TBE protein was confirmed using a transient transfection system in which a recombinant FLAG-tagged hRDH-TBE protein was expressed in COS cells. The polyclonal antibody recognizes one particular band of ~35 kDa that is specific to FLAG-hRDH-TBE transfected cells (Fig. 6A). The anti-FLAG antibody M5 also recognizes this 35 kDa band. The 35 kDa band is not recognized by preimmune serum, and it is abolished after preincubation of anti-hRDH-TBE antiserum with the peptide antigen (data not shown).

The anti-hRDH-TBE antiserum was then used to immunohistochemically stain primate tracheal and human esophageal tissue sections (Fig. 6B). For the tracheal tissue section, positive stain was seen on most of the ciliated cells, whereas there was no stain for other epithelial cell types such as goblet cells or the cells underlying the epithelia. These observations corroborated the above in situ hybridization study. The esophagus showed staining of the superficial layers of the tissue, but not the lamina propria. These observations are consistent with tissue Northern blot analysis. Incubation of either trachea or esophagus sections with preimmune serum did not produce any specific staining (data not shown).

**Characterization of hRDH-TBE Function in the Metabolism of All-trans-Retinol**—To elucidate the putative function of this newly found hRDH-TBE gene, the metabolism of all-trans-retinol in primary human TBE cultures was analyzed by an HPLC method as described under “Experimental Procedures” (Fig. 7). Despite the capability of resolving retinaldehyde by HPLC based on the retinoid standards used, we were unable to detect retinaldehyde in any of our cell extracts. In at least one other report, the retinaldehyde intermediate in the metabolism of retinol to retinoic acid was not detectable by HPLC (10). Further, retinaldehyde is notably difficult to extract and quantify because of its extreme reactivity (50, 51). We therefore chose retinoic acid as the end point of the assay, as the product of the reversible oxidation of retinol to the short-lived intermediate retinaldehyde, which is then further oxidized irreversibly to RA. As shown in the HPLC profile, several RAs as the metabolites of all-trans-retinol in primary human TBE cultures could be demonstrated.

Using this approach, the metabolism of all-trans-retinol in primary human TBE cells was studied and compared in two culture systems that were maintained continuously in either all-trans-RA-supplemented- or -deprived culture medium. As we have demonstrated before, growth of TBE cells with supplementary all-trans-RA elevates the amount of hRDH-TBE transcript (Figs. 5 and 7A). Western blotting of proteins extracted from cultures grown without and with supplementary all-trans-RA demonstrated that the levels of hRDH-TBE protein are also elevated (Fig. 7B). HPLC chromatographs showed the presence of several isomers of RA after retinol treatment of retinoid acid supplemented TBE cells (Fig. 7C). As shown in Fig. 7, C and D, all-trans-RA levels were elevated in cultures that had been maintained long-term in all-trans-RA-supple-
COS cells gained the ability to metabolize retinol only after putative retinol dehydrogenase gene expression. This was demonstrated that human TBE cells were able to metabolize retinol even after the all-trans-retinol addition. These results suggest that the newly found hRDH-TBE gene is probably a major player in the regulation of all-trans-retinol metabolism in various epithelial tissues, including that of the respiratory system.

There are several lines of evidence to support the putative role of this new hRDH-TBE gene in the metabolism of retinol. One line of evidence is the conservation of motifs that are diagnostic indicators of SDR gene family membership. The first motif sequence, GXXXGXG at hRDH-TBE amino acid residues 36–42, is a structural feature known as the Rossmann fold (52) that is important in cofactor binding. The XXXX sequence at hRDH-TBE amino acid residues 176–180 has been suggested as the putative active site of dehydrogenase/reductase enzymes. Whereas the functions of the sequences LXXNAG at hRDH-TBE amino acid residues 109–114, and PG at hRDH-TBE amino acid residues 206–207 are still unknown, these two motifs are quite conserved in the SDR gene family. In addition, the phylogenetic tree analysis demonstrated that the gene is well fitted into the SDR gene family with a close association to various retinoid-associated SDR genes, such as the eye’s 11-cis-retinol dehydrogenase and multiple liver dehydrogenases.

An additional line of evidence to support the putative role of this gene product in retinol metabolism is from a correlation study. We have observed a correlation of the metabolism of retinol in culture with the expression of this gene product. Using all-trans-RA to stimulate the expression of the hRDH-TBE gene in culture, we also observed an enhanced retinol metabolism in this culture. However, retinoic acid is known to induce the expression of many genes in cultured human TBE cells; a gene such as alcohol dehydrogenase IV (ADH4) could also have been induced and could have been responsible for retinol metabolism under the experimental conditions used. Thus, the correlated data may not be sufficient to support the conclusion that hRDH-TBE functions as a retinol dehydrogenase. To further confirm the functional role of this hRDH-TBE gene product in the retinol metabolism, we transfected COS cells with an expression construct of FLAG-hRDH-TBE. COS cells lack the expression of the hRDH-TBE gene under the experimental conditions that were used (Fig. 8A). After transfection, COS cells were able to metabolize all-trans-retinol into RA. The control transfection with the parent vector pFLAG-CMV2 DNA failed to confer the retinol metabolizing activity. These results suggest that this newly found hRDH-TBE gene is potentially a major enzyme involved in the metabolism of retinol in airways. Further studies with purified recombinant hRDH-TBE protein will help to resolve the functional nature of this new hRDH-TBE gene.

Despite having high homology to retinoid-associated SDR gene family members, there are some differences between this new hRDH-TBE gene and the rest of this gene subfamily. All of the retinoid-associated SDR gene family members have a conserved N-terminal 115 amino acid sequence (31), whereas the hRDH-TBE gene does not share this N-terminal sequence. Additionally, all of the retinoid-associated SDR gene family members have much higher homology to each other (≥80%) than hRDH-TBE does to any of them (best homology is only 45% at the amino acid level). It is possible that hRDH-TBE represents a new class of retinol dehydrogenase that is differen-
FIG. 7. Metabolism of all-trans-retinol in cultured human TBE cells. A, Northern blots of RNA hybridized with hRDH-TBE DNA and 18 S rDNA probes. Lane 1, no all-trans-RA pretreatment; lane 2, 30 nM all-trans-RA pretreatment for 9 days prior to harvest. Lane 3, no DNA; lane 4, pFLAG CMV 2 (empty expression vector). B, Western blots of protein extracts from all-trans-RA-treated and unsupplemented TBE cells hybridized with rabbit polyclonal antibody-hRDH-TBE serum (1:2000 dilution). Lanes as in A. The arrow indicates the hRDH-TBE protein. C, representative HPLC chromatographs of samples pretreated with all-trans-RA and incubated without (upper trace) or with (lower trace) all-trans-retinol. The positions of retinoid standards are shown as arrows pointing to specific retention times. a, 13-cis-RA; b, 9-cis-RA; c, all-trans-RA; d, 4-hydroxyphenyl retinamide (4-HPR), which was used as the internal standard; e, all-trans-retinol; f, all-trans-retinaldehyde. D, amount (in pmol/mg protein) of all-trans-RA in all the sample groups, calculated from areas under peaks in chromatographs. Group 1, no all-trans-RA pretreatment, no all-trans-retinol; group 2, no all-trans-RA pretreatment, 3 μM all-trans-retinol; group 3, 10⁻⁷ M all-trans-RA pretreatment, no all-trans-retinol; group 4, 10⁻⁷ M all-trans-RA pretreatment, 3 μM all-trans-retinol.

FIG. 8. Metabolism of all-trans retinol in transiently transfected COS cells. A, COS cells were transiently transfected with either no DNA, pFLAG CMV 2 (empty expression vector), or pFLAG-hRDH-TBE. Protein extracts of the transiently transfected COS cells were analyzed by Western hybridization using the rabbit polyclonal antibody-hRDH-TBE serum (1:2000 dilution). Lane 1, no DNA; lane 2, pFLAG CMV 2; lane 3, pFLAG-hRDH-TBE. B, amount (in pmol/mg protein) of all-trans-RA in each sample group, calculated from areas under peaks in chromatographs. Group 1, no DNA; group 2, pFLAG CMV 2; group 3, pFLAG-hRDH-TBE.

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