The Retinol Dehydrogenase Rdh10 Localizes to Lipid Droplets during Acyl Ester Biosynthesis*

Weiya Jiang and Joseph L. Napoli

From the Department of Nutritional Sciences and Toxicology, Graduate Program in Metabolic Biology, University of California, Berkeley, California 94720

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Background: Rdh10 is a short chain dehydrogenase essential for retinoic acid biosynthesis.

Results: Rdh10, a membrane-associated enzyme, localizes with lipid droplets during acyl ester biosynthesis along with cellular retinol binding-protein, type 1 and lecithin:retinol acyltransferase.

Conclusion: Lipid droplets serve as a site of retinoid homeostasis.

Significance: These results provide insight into lipid droplet function and retinol metabolism.

Rdh10 catalyzes the first step of all-trans-retinoic acid biosynthesis, conversion of retinol into retinal. We show that Rdh10 associates predominantly with mitochondria/mitochondrial-associated membrane (MAM) in the absence of lipid droplet biosynthesis, but also locates with lipid droplets during acyl ester biosynthesis. Targeting to lipid droplets requires the 32 N-terminal residues, which include a hydrophobic region followed by a net positive charge. Targeting to mitochondria/MAM and/or the stability of Rdh10 require both the N-terminal and the 48 C-terminal hydrophobic residues. Rdh10 behaves similarly to cellular retinol-binding protein, type 1, which also localizes to mitochondria/MAM before lipid droplet synthesis, and associates with lipid droplets during acyl ester synthesis (Jiang, W., and Napoli, J. L. (2012) Biochem. Biophys. Acta 1820, 859–8692). LRAT, an ER protein, also associates with lipid droplets upon acyl ester biosynthesis. Colocalization of Rdh10, Crbp1, and LRAT on lipid droplets suggests a metabolon that mediates retinol homeostasis.

During low or normal vitamin A intake, retinol dehydrogenases (Rdh)2 of the short chain dehydrogenase/reductase gene (SDR) family catalyze the first step of all-trans-retinoic acid (atRA) biosynthesis from retinol (vitamin A), the conversion of retinol into retinal (1, 2). Although multiple retinoid-recognizing Rdh/SDR have been identified, only four have been associated unequivocally with physiological atRA production and vitamin A-supported systemic functions (3). These include Rdh1, Dhrs9, Rdhe2, and Rdh10. Surprisingly, these Rdh often occur in the same cell types, suggesting independent regulation, perhaps generating specific pools of atRA to support discrete vitamin A-dependent processes (4, 5). Emerging data foster this notion. Embryos express Rdh1 starting E7.5 with intensity increasing 40-fold during embryogenesis (6, 7). Yet, Rdh1-null mice are born apparently normal under laboratory conditions. Starting early in life, however, Rdh1-null mice grow heavier as a result of increased adiposity, such that at 33 weeks old Rdh1-null male mice fed rodent chow with a normal fat content (i.e. not a high fat diet) weight 37% more than WT, if dietary vitamin A is not copious (7). In contrast, mouse Dhrs9 has not been knocked out, but the zebrafish ortholog of Dhrs9 contributes to atRA production that supports gut development (8). In mammalian cells, Dhrs9 seems to function as a tumor suppressor, consistent with its poor expression in colon cancer cell lines accompanied by insubstantial atRA biosynthesis, relative to normal colon cells (9). In the frog, overexpression of Rdh2 produces posteriorization defects, similar to those caused by atRA toxicity, whereas substantial knock-down causes embryonic lethality (10). Rdh10 was identified initially as a visual cycle enzyme (11). Subsequently, Rdh10 was correlated with multiple sites of atRA biosynthesis in the mouse embryo (12). The Rdh10-null mouse is embryonic lethal, and hypomorphs fail to develop a normal cortex, because of decreased but not absent atRA (13–16). Rdh10 also produces atRA to regulate genes in human epidermis (17). These cumulative data indicate that although Rdh10 contributes essential pools of atRA during embryogenesis and postnatally, it is not the only essential Rdh, because Rdh10-null mice develop until nearly E12.5, embryos do not exhibit phenotypes consistent with total atRA deficiency, and other Rdh regulate specific vitamin-dependent processes postnatally. These data support the notion that multiple Rdh generate atRA targeted to specific vitamin A-dependent processes.

Many Rdh associate with the smooth ER, oriented toward the cytoplasm (18–21). To provide insight into the contribution of Rdh expression loci to function, we undertook a detailed study of the subcellular expression loci of Rdh10 and compared the results to Rdh1, a well established ER enzyme (19, 21). We found that Rdh10 distributes differently than Rdh1. Rdh10 has broader expression loci than Rdh1 in the absence of lipid droplet synthesis, including mitochondria/mitochondrial-associated membranes (MAM), and in contrast to Rdh1, Rdh10
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| TABLE 1  | Primers use to create constructs | Sequences |
|----------|----------------------------------|-----------|
| Rdh10-GFP | Forward                          | 5’-ATGAATTCATGAAAATCCTGTTTCTTCC  |
|          | Reverse                          | 5’-TCGAATCCAGATCTATCTTTTTCTTTCT    |
| Rdh1-GFP | Forward                          | 5’-ATGAATTCATGGAAGCTTCCTCTGTATGCAC  |
|          | Reverse                          | 5’-TCGAATCCAGATCTATCTTTTTCTTTCT    |
| Rdh10   | Forward                          | 5’-ATGAATTCATGAAAATCCTGTTTCTTCC  |
|          | Reverse                          | 5’-TCGAATCCAGATCTATCTTTTTCTTTCT    |
| ΔN-terminal Rdh10 | Forward  | 5’-ATGAATTCATGGAAGCTTCCTCTGTATGCAC  |
|          | Reverse                          | 5’-TCGAATCCAGATCTATCTTTTTCTTTCT    |
| ΔC-terminal RDH10 | Forward  | 5’-ATGAATTCATGAAAATCCTGTTTCTTCC  |
|          | Reverse                          | 5’-TCGAATCCAGATCTATCTTTTTCTTTCT    |
| ΔT-RDH10 | Forward                          | 5’-ATGAATTCATGAAAATCCTGTTTCTTCC  |
|          | Reverse                          | 5’-TCGAATCCAGATCTATCTTTTTCTTTCT    |
| Rdh10-t-GFP | Forward  | 5’-ATGAATTCATGGAAGCTTCCTCTGTATGCAC  |
|          | Reverse                          | 5’-TCGAATCCAGATCTATCTTTTTCTTTCT    |
| Rdh10-S-GFP | Forward  | 5’-ATGAATTCATGAAAATCCTGTTTCTTCC  |
|          | Reverse                          | 5’-TCGAATCCAGATCTATCTTTTTCTTTCT    |

locates partially to lipid droplets during acyl ester biosynthesis. Rdh10 associated with the lipid droplet fraction has greater specific enzymatic activity than Rdh10 associated with the ER or the MAM. We also identify the N-terminal residues that are necessary, and those that are both necessary and sufficient for lipid droplet targeting. The data provide further insight into the necessary and sufficient for atRA biosynthesis, the function of Rdh10, and differences among Rdh.

**EXPERIMENTAL PROCEDURES**

**Antibodies**—Rabbit anti-GFP was purchased from Invitrogen (catalog number A-11122). Mouse anti-cytochrome c (catalog number 556433) and mouse anti-calreticulin (catalog number 612136) were purchased from BD Biosciences. Guinea pig anti-ADRP was purchased from Fitzgerald Industries International (catalog number RDI-PROGP40). Secondary antibodies were purchased from Sigma.

**Cell Culture**—COS7 cells were cultured in DMEM with 10% fetal bovine serum in 24-well plates. For confocal fluorescence microscopy imaging, cells were grown on 12-mm glass coverslips. A solution of oleic acid bound to bovine serum albumin (catalog number 612136) were purchased from BD Biosciences. Guinea pig anti-ADRP was purchased from Fitzgerald Industries International (catalog number RDI-PROGP40). Secondary antibodies were purchased from Sigma.

**Expression Constructs and Cell Transfection**—The mouse Rdh10 coding region was obtained from ATCC, IMAGE number 4923005. Constructs were cloned into pGFP2-N2 as a GFP fusion at the C-terminal using the EcoRI and BamHI sites and the primers shown in Table 1. The Rdh1(1–22)-GFP construct was obtained as described (19, 21). Cells were transfected with 0.1–0.2 μg of construct in Lipofectamine, i.e. 4–12-fold less than recommended to reduce expression levels.

**Immunofluorescence Microscopy**—Cells were fixed with 4% paraformaldehyde for 15 min at room temperature. Cells were washed for 5 min three times with PBS. Fixed cells were permeabilized with 0.1% Triton X-100 for 5 min. Lipid droplets were stained with Nile Red. Nuclei were stained with DAPI (Invitrogen). Live mitochondria staining was done according to the manufacturer’s protocol using MitoTracker Red CMXRos (Invitrogen). RE autofluorescent images were taken with an excitation wavelength of 350 nm and emission was collected from 610 to 700 nm, wavelengths with diminished GFP emission. A Carl Zeiss LSM 510 Meta laser scanning confocal microscope with a ×100 objective was used to take images.

**Subcellular Fractionation**—Fractionations were done at 4 °C by a published procedure (25). Three 150-mm dishes of cells were combined according to the protocol in Ref. 22. Retinol-free medium was prepared by irradiating commercial medium for 60 min. The crude ER fraction was loaded onto a 0.25, 1.0, and 1.7 M sucrose gradient and centrifuged at 15,000 × g for 10 min. Crude mitochondria (pellet) and ER fractions (supernatant) were separated by centrifuging at 15,000 × g for 10 min. Crude mitochondria were loaded onto a 0.25, 1.0, and 1.7 M sucrose gradient and centrifuged at 40,000 × g for 30 min. Purified mitochondria were isolated from the 1.0/1.7 M interface. MAM were isolated from the 1.0/0.25 M interface and precipitated at 105,000 × g for 60 min. The crude ER fraction was loaded onto a 0.25/1.0 M sucrose gradient and centrifuged at 40,000 × g for 30 min. ER and cytosol fractions were collected above the 1.0 M
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FIGURE 1. Subcellular expression of Rdh10 versus Rdh1. A–C, Rdh10-GFP expression in COS7 cells also expressing Dgat2 to augment acyl ester biosynthesis. Cells were transfected with 0.1 μg of Rdh10-GFP and 0.1 μg of Dgat2. Left to right, Rdh10-GFP (green), Mitotracker (red, A and B, only), lipid droplets (red, C only). A. no oleate was added to the medium. B and C, cells were incubated 5 h with 400 μM oleate starting 24 h post-transfection. D and E, COS7 cells were transfected with 0.1 μg of Rdh1(1–22)-GFP. Left to right, Rdh1(1–22)-GFP (green); lipid droplets (red); merge. D, no oleate was added to the medium. E, cells were incubated with oleate. F, subcellular distribution of Rdh10 before and after incubation of COS7 cells with oleate. Western blotting of subcellular fractions: T, total homogenate; ER, microsomal fraction; M, mitochondrial fraction; MAM, mitochondria-associated membranes; L, lipid droplet fraction. The ER pellet was collected after 105,000 × g centrifugation for 60 min. The lipid droplet fraction was isolated as a floating white layer at the top. Lipid droplets were delipidated by sonicating the lipid droplet mixture in 5% SDS for 1 h at 37 °C. Proteins were analyzed by SDS-10% PAGE.

RESULTS

Rdh10 and Rdh1 Do Not Overlap in Subcellular Locii—To determine subcellular localization of Rdh10, we applied confocal fluorescence microscopy to COS7 cells transfected to express a full-length Rdh10 with a C terminus GFP label (Rdh10-GFP). We also transfected cells with Dgat2, which catalyzes the final step of triglyceride synthesis, to maximize lipid droplet formation (27). In the absence of oleate to stimulate lipid droplet formation from Dgat2, Rdh10 was localized with the mitochondria/MAM marker, and to some extent with the ER (Fig. 1A). In cells incubated with oleate to stimulate lipid droplet formation, Rdh10 had relatively weak expression in the mitochondria/MAM and the ER compared with cells not synthesizing lipid droplets (Fig. 1, B and D). In this state, Rdh10 showed intense expression in rings that did not overlap with the mitochondria/MAM. The rings of Rdh10 surround lipid droplets during acyl ester biosynthesis. Similar results were obtained in cells transfected with Rdh10-GFP in the absence of Dgat2 after a 24-h incubation with oleic acid (not shown), although lipid droplet synthesis was not as extensive.

Expression of Rdh10 was then compared with Rdh1. We used the first 22 residues of Rdh1 labeled with GFP (Rdh1(1–22)-GFP). The first 22 residues of Rdh1 are necessary and sufficient to anchor Rdh1 or GFP in the ER facing the cytoplasm (19, 21). Consistent with previous results Rdh1(1–22)-GFP was distributed throughout the ER (Fig. 1E, F). Rdh1 did not localize extensively around lipid droplets, either in the absence or presence of oleate. In a small fraction of cells (<10%), however, Rdh1(1–22)-GFP showed lipid droplet association similar to Rdh10.

To confirm localization of Rdh10, we transfected COS7 cells with Rdh10-GFP, Western blotted the subcellular fractions, and compared localization to the ER and mitochondria markers, calreticulin and cytochrome c, respectively (28, 29). In the absence of induced lipid droplet synthesis, Rdh10-GFP was enriched in mitochondria, and to a lesser extent associated with droplets. COS7 cells were depleted of lipid droplets by 6 h incubation with serum-free medium before transfection (−oleate). Another group of three 150-mm plates was incubated with oleic acid overnight (+oleate). Five μg of protein were loaded in each lane. Five of 60 μl of the lipid droplet fraction were loaded. Oleate stimulated cell proliferation, such that the total protein increased from 17 to 33 mg after oleate treatment, and mitochondrial protein increased from 0.29 to 0.4 mg. Calreticulin (cal) and cytochrome c (cyt) served as markers of the ER and mitochondria, respectively. G. HepG2 and COS7 cells were transfected with Rdh10-GFP (30 μg of plasmid/150-mm plate, 3 plates/group) and treated with oleate. Lipid droplets were isolated by centrifugation. Lipid droplet-associated proteins were resuspended in 60 μl of lysis buffer (0.25 M sucrose in 10 mM HEPES, 1 mM EDTA). An aliquot (5 of 60 μl) containing ~0.01 mg of TAG was loaded onto the gel. Protein could not be quantified in the lipid droplet fraction: L, lipid droplet fraction; T, total cell lysate. In other fractions, 5 μg of protein were loaded.
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TABLE 2
Distribution of Rdh10 enzymatic activity
Subcellular fractions were isolated by differential centrifugation of COS7 cells transfected with Rdh10. Data are mean ± S.E., n = 3. The medium either had no added oleate (−), or cells were incubated 24 h with 400 μM oleic acid (+) to stimulate lipid droplet formation.

| Subcellular fraction | − Oleate (pmol/min/mg protein) | + Oleate (pmol/min/mg protein) |
|----------------------|-------------------------------|-------------------------------|
| ER                   | 34 ± 0.6 (58)                 | 29 ± 5 (36)                   |
| Mitochondria         | 43 ± 1 (14)                   | ND†                          |
| MAM                  | 87 ± 0.6 (28)                 | 132 ± 27 (32)                |
| lipid droplets       | None† (ND)                    | >200† (32)                   |

† ND, net activity not detectable.
† None, lipid droplet protein not quantifiable.
† Very active, but protein too low to make precise determination.

the MAM fraction, but relatively little localized to the ER fraction, and lipid droplets were too few to isolate (Fig. 1F). Oleate treatment not only simulated lipid droplet synthesis, but also induced cell proliferation: the latter is a well known effect of oleate on mammalian cells (30–37). After lipid droplet synthesis stimulated by oleate, mitochondrial protein was increased from 0.29 to 0.4 mg. The same size aliquot was analyzed on the gel, resulting in a smaller proportion of total protein for the oleate-treated cells. Thus, Rdh10 was decreased ∼30% in the mitochondria fraction and was associated (partially) with lipid droplets.

Next, we compared isolated Rdh10 expression in the total cell lysate versus the lipid droplet fraction in COS7 and HepG2 cells treated with oleate by Western blotting (Fig. 1G). The PAT family lipid droplet binding-protein ADRP (now known as perilipin 2) was used to confirm the presence of lipid droplets (38). Subcellular fractions confirmed association of Rdh10 with lipid droplets.

**Distribution of Rdh10 Activity**—We measured Rdh activity in subcellular fractions and lipid droplets of COS7 cells transfected with Rdh10, in the absence and presence of oleate. Cells not exposed to oleate had the highest Rdh10-specific enzyme activity (pmol of retinal formed/min/mg of protein) in the MAM fraction, with ∼40–50% lower activities in the mitochondria and ER fractions (Table 2). Although activity was highest in the MAM fraction, indicating a higher concentration of Rdh10 in the MAM, a larger amount of Rdh10 was associated with the ER (% enzyme units recovered), perhaps reflecting the extent of the ER relative to mitochondria and MAM. In the absence of incubating with oleic acid, lipid droplets were too few to isolate and analyze. In the presence of lipid droplets, stimulated by treating cells with oleate, both the MAM and lipid droplets increased in specific activity and amount of Rdh10 (enzyme units), largely at the expense of mitochondria and ER. The most marked increase was in the lipid droplets. These data show that Rdh10 activity and protein associates with lipid droplets upon lipid droplet formation.

**Rdh10-GFP Localizes with Both RE- and TAG-Rich Lipid Droplets**—To test whether Rdh10 forms rings around lipid droplets during biosynthesis of retinyl esters (RE), COS7 cells were co-transfected with Rdh10-GFP and LRAT. LRAT serves as the major acyltransferase that catalyzes RE formation, and the only acyltransferase known that interacts with holo-Crbp1 to acquire retinol (39–43). RE-rich lipid droplets in cells incubated with retinol were visualized by RE autofluorescence.

Rdh10 surrounded RE-rich lipid droplets (Fig. 2, A–C). To confirm whether Rdh10 associates with TAG-containing lipid droplets in the absence of RE, we irradiated medium supplemented with 10% FBS to eliminate retinol, which left only trace amounts (50 nm by HPLC). COS7 cells were transfected with Rdh10-GFP and DGAT2, and incubated 5 h with 400 μM oleic acid in retinol-depleted medium: D, nuclei (blue); B, Rdh10-GFP (green); C, lipid droplets (red); E, merge. Bars represent 5 μm.

**The N Terminus and Flanking Positive Charges Target Rdh10 to Lipid Droplets**—The Protoscale and SOSUI programs (ca. expasy.org) predicted two possible transmembrane domains in Rdh10, at the N terminus and near the C terminus (Fig. 3A). We constructed three Rdh10 mutants lacking the N-terminal transmembrane residues 1–26 (Rdh10-N-GFP), or the C-terminal transmembrane residues 293–341 (Rdh10-C-GFP), or...
both (Rdh10-NC-GFP) (Fig. 3B). Constructs lacking the N-terminal or both the N and C termini did not form rings around lipid droplets, but instead displayed diffuse patterns. Constructs without the C-terminal domain continued to form rings around lipid droplets, albeit with areas of localized concentrations (Fig. 3, C-E). Rdh10-N-GFP, lacking both N- and C-terminal residues did not localize with lipid droplets and localized differently than the Rdh10-N-GFP, which lacked only N-terminal residues, indicating that both the N- and C-terminal regions participate in directing loci of Rdh10 expression.

None of the deletion constructs extensively co-localized with mitochondria/MAM (absence of incubation with oleic acid) by confocal microscopy, suggesting major mitochondria/MAM targeting requires both putative transmembrane regions (Fig. 3B). Western blot, however, revealed that the deletion mutants were not expressed as strongly as Rdh10-GFP and/or were unstable. Western blot analysis also indicated that the N- and C-terminal deletion mutants, Rdh10-N-GFP and Rdh10-C-GFP, were associated partially with the mitochondria/MAM, but the double deletion mutant, Rdh10-NC-GFP, did not (Fig. 4D). These data can be interpreted as Rdh10 mutants partially re-localizing during homogenization and centrifugation to mitochondria/MAM. In agreement with the data of Fig. 3, C-E, Rdh10-C-GFP was the only one of the three deletion mutants that associated with lipid droplets (Fig. 4, C and D).

To assess further the contribution of the N terminus to lipid droplet association, we fused a shorter and a longer leader of Rdh10 to GFP (Table 3). The shorter construct, Rdh10-S-GFP, contained the N-terminal transmembrane domain (residues 1–26) that was deleted in Rdh10-N-GFP. The longer one extended the N terminus to residue 33 (Rdh10-L-GFP), i.e. included the flanking residues and increased the net positive...
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**TABLE 3**

N-terminal sequences of Rdh10 and Rdh1 mutant constructs

Underlining indicates putative transmembrane regions predicted by the SOSUI program. Positively (red) and negatively (blue) charged residues are indicated. Constructs were linked to GFP at their C termini. Rdh10-L-GFP has the putative transmembrane region and flanking charged residues of Rdh10. Rdh10-S-GFP terminates after the putative transmembrane region, before the flanking charged residues. Rdh1(1–22)-GFP was used for reference.

| construct     | N-terminal sequence             | net positive charge |
|---------------|---------------------------------|---------------------|
| Rdh10-L-GFP   | MNIVVEFYYTFXVLWAFVLQAARWLPRKEX-GFP | 3                   |
| Rdh10-S-GFP   | MNIVVEFYYTFXVLWAFVLQAARWLGFP     | 1                   |
| Rdh1-GFP      | MWTYVLAVGLYLULRFRGQ-GFP          | 2                   |

**FIGURE 5.** The N-terminal transmembrane domain and flanking net positive charges target Rdh10 to lipid droplets. COS7 cells were transfected with 0.2 μg of vector and incubated 7 h with 400 μM oleic acid: A, Rdh10-L-GFP; B, Rdh10-S-GFP; C, Rdh1-GFP; D and E, expanded insets from A–C, respectively; left to right, GFP constructs (green); lipid droplets (red); merge. The bar in A shows 5 μm.

charge. We compared these sequences to the first 22 residues of the Rdh1 leader sequence fused to GFP, because this sequence is necessary and sufficient to direct GFP to the ER (19, 21). The longer sequence of Rdh10 targeted GFP to lipid droplets, but showed higher cytoplasmic background than Rdh10-GFP, suggesting that additional residues enhance lipid droplet targeting (Fig. 5, A and D). The shorter version produced a more diffuse pattern, with limited lipid droplet targeting (Fig. 5, B and E). Less than 5% of cells showed slight localization in rings surrounding lipid droplets, whereas all transfected cells had high background throughout the cytoplasm. In comparison, the Rdh1 leader sequence targeted GFP to the ER network and not to lipid droplets (Fig. 5, C and F).

**DISCUSSION**

Confocal microscopy and subcellular fractionation revealed the strongest association of Rdh10 with mitochondria, lesser association with MAM, and a much lower concentration in the ER, in the absence of lipid droplet synthesis. Also in the absence of lipid droplet biosynthesis, enzyme activity, was distributed among mitochondria, MAM, and ER. Rdh10 activity in the ER, disproportionate to the minimal association of Rdh10 protein in the ER (confocal and Western), probably reflects contamination of the ER by a highly active and hydrophobic enzyme. Incubation with either retinol or oleate induced association of Rdh10 with lipid droplets, verified by co-association with ADRP (perilipin 2). Rdh10 activity, however, decreased disproportionately in mitochondria although substantial Rdh10 protein remained with mitochondria. Mitochondria association of the Rdh10 protein during acyl ester biosynthesis with undetectable activity suggests that mitochondria harbor an inactive form, and possibly that association with MAM and lipid droplets enhances catalytic activity.

MAM consist of subdomains of the ER that engage physically with mitochondria outer membranes in various degrees, including a single by-pass contacting a limited portion, more extensive contact engaging a considerable portion, and total envelopment (44). MAM serve multiple functions, including regulating communication between the ER and mitochondria, lipid transport, control of cholesterol and neutral lipid biosynthesis and metabolism, and lipid droplet formation. For example, acyl-CoA:cholesterol O-acyltransferase and DGAT2 are among the enzymes that associate with mitochondrial/MAM (45, 46). Occurrence of Rdh10 in MAM places this Rdh in a subcellular locus active in lipid metabolism.

Lipid droplets emerge from the ER/MAM (47, 48). MAM are in contact with mitochondria, and Rdh10 is associated with MAM and mitochondria before lipid droplet synthesis, and with lipid droplets when they form. Placed in this context, migration of Rdh10 from mitochondria/MAM seems a reasonable hypothesis.

Recent insights into the structure and functions of lipid droplets indicate that they serve as complex organelles with multiple functions, not just as depositories for acyl ester storage (49, 50). The proteome of the lipid droplet includes multiple varieties of proteins, in addition to perilipins, lipases, and acyltransferases, likely partially typical of specific cell types (51–53). These include SDR that catalyze steroid metabolism, such as isoforms of 3β- and 17β-hydroxysteroid dehydrogenases, indicating that the metabolism of lipids in addition to acyl esters occurs on the surfaces of lipid droplets. The presence on lipid droplet surfaces of SDR that catalyze retinoid metabolism indicates lipid droplet-associated metabolism of retinoids. In fact, Rdh10, the retinal reductase Dhrs3 (also known as retSDR1), and the retinoid/sterol oxidoreductase RDHE2 (also known as RetSDR2,
17βHSD11) have been identified in the proteomes of lipid droplets (51, 53, 54). Dhrs3 reduces retinal in the eye and metabolizes retinal derived from β-carotene cleavage (55, 56). These data suggest that the coordinated actions of Dhrs3 and Rdh10 manage retinoid homeostasis to balance RE and RA formation on the lipid droplet, where there occurs direct access to substrates.

Crbp1 chaperones RE biosynthesis catalyzed by LRAT, and retinal biosynthesis catalyzed by Rdh (40, 41, 57–64). Before acyl ester biosynthesis, Crbp1 localizes with MAM and LRAT distributes throughout the ER with some surrounding the Crbp1 (65). During acyl ester biosynthesis, Crbp1 remains with MAM, and Crbp1, MAM, and LRAT co-localize on the surfaces of lipid droplets. LRAT associated with lipid droplets is more active relative to the ER. The ratio apo-Crbp1/holo-Crbp1 controls the relative rates of RE hydrolysis and biosynthesis (66, 67). Therefore, the cumulative data suggest coordinated function of Crbp1, LRAT, and Rdh10 on lipid droplets to store retinol as RE and/or to mobilize retinol to generate retinyl for atRA biosynthesis.

Substantial Rdh10 targeting to lipid droplets required the N-terminal hydrophobic amino acid residues (confocal microscopy), but these residues were insufficient to direct GFP to lipid droplets. The constructs with GFP showed that the charged residues at the end of the Rdh10 N-terminal hydrophobic sequence, with a net positive charge of three, also were necessary for lipid droplet association. As a comparison, Rdh1 has positively charged residues at the end of its N-terminal hydrophobic sequence, with a net positive charge of two, but targets to the ER (19, 21). It is difficult to discern how these similar primary sequences direct the two SDR to different loci. Nevertheless, a situation similar to Rdh10 occurs with LRAT. Both the N-terminal hydrophobic sequence and the charged amino acid residues at the end of the N terminus are necessary and sufficient to target LRAT to lipid droplets. In distinction to its lipid droplet targeting, Rdh10 targeting to mitochondria/MAM and/or the stability of Rdh10 require both the N- and C-terminal transmembrane regions.

In contrast to Rdh10, Rdh1 associates with the ER (19, 21, 57, 59). Although Rdh1 expression increases 40-fold during embryogenesis, Rdh1 null mutants show no obvious signs of impaired development, but have abnormally high post-weaning adiposity (7). Rdh10-null mice, in contrast, die in midgestation with multiple, but not complete, dysfunctions in atRA-dependent processes (13, 14, 16, 68). These two distinct phenotypes, along with different subcellular loci in the same cells, are consistent with the hypothesis that individual Rdh isoforms generate discrete pools of atRA to serve specific vitamin A-dependent functions.

In summary, Rdh10 associates with lipid droplet surfaces upon acyl ester biosynthesis. In this regard, Rdh10 behaves similarly to Crbp1, which localizes to mitochondria/MAM before lipid droplet synthesis, and migrates to the surfaces of lipid droplets during acyl ester synthesis. Both Crbp1 and Rdh10 are distinct from LRAT in initial locus, a resident ER protein in the absence of acyl ester biosynthesis, which migrates to the surfaces of lipid droplets. The loci and behavior of Rdh10, colocalizing with Dhrs3, LRAT, and Crbp1, suggests a metabolon that controls retinol homeostasis through regulating RE and RA producing substrates and delivering retinoids directly to/from lipid droplets.

REFERENCES

1. Napoli, J. L. (1996) Biochemical pathways of retinoid transport, metabolism, and signal transduction. *Clin. Immunol. Immunopathol.* **80**, 552–62

2. Belyaeva, O. V., and Kedischvili, N. Y. (2006) Comparative genomic and phylogenetic analysis of short-chain dehydrogenases/reductases with dual retinol/sterol substrate specificity. *Genomics* **88**, 820–830

3. Napoli, J. L. (2012) Physiological insights into all-trans-retinoic acid biosynthesis. *Biochim. Biophys. Acta* **1821**, 152–167

4. Zhai, Y., Higgins, D., and Napoli, J. L. (1997) Coexpression of the mRNAs encoding retinol dehydrogenase isozymes and cellular retinol-binding protein. *J. Cell. Physiol.* **173**, 36–43

5. Wang, C., Kane, M. A., and Napoli, J. L. (2011) Multiple retinol and retinal dehydrogenases catalyze all-trans-retinoic acid biosynthesis in astrocytes. *J. Biol. Chem.* **286**, 6542–6553

6. Zhang, M., Chen, W., Smith, S. M., and Napoli, J. L. (2001) Molecular characterization of a mouse short chain dehydrogenase/reductase active with al-trans-retinol in intact cells, mRDH1. *J. Biol. Chem.* **276**, 44083–44090

7. Zhang, M., Hu, P., Krois, C. R., Kane, M. A., and Napoli, J. L. (2007) Altered vitamin A homeostasis and increased size and adiposity in the rdh1-null mouse. *FASEB J.* **21**, 2886–2896

8. Nadauld, L. D., Sandoval, I. T., Chiester, S., Yost, H. J., and Jones, D. A. (2004) Adenomatous polyposis coli control of retinoic acid biosynthesis is critical for zebrafish intestinal development and differentiation. *J. Biol. Chem.* **279**, 51581–51589

9. Nadauld, L. D., Phelps, R., Moore, B. C., Eisinger, A., Sandoval, I. T., Chiester, S., Peterson, P. W., Manos, E. J., Sklow, B., Burt, R. W., and Jones, D. A. (2006) Adenomatous polyposis coli control of C-terminal binding protein-1 stability regulates expression of intestinal retinol dehydrogenases. *J. Biol. Chem.* **281**, 37828–37835

10. Belyaeva, O. V., Lee, S. A., Adams, M. K., Chang, C., and Kedischvili, N. Y. (2012) Short chain dehydrogenase/reductase rdh2 is a novel retinol dehydrogenase essential for frog embryonic development. *J. Biol. Chem.* **287**, 9061–9071

11. Wu, B. X., Chen, Y., Chen, Y., Fan, J., Rohrer, B., Crouch, R. K., and Ma, J. X. (2002) Cloning and characterization of a novel all-trans-retinol short-chain dehydrogenase/reductase from the RPE. *Invest. Ophthalmol. Vis. Sci.* **43**, 3365–3372

12. Cammas, L., Romand, R., Fraulob, V., Mura, C., and Dollé, P. (2007) Expression of the murine retinol dehydrogenase 10 (Rdh10) gene correlates with many sites of retinoid signalling during embryogenesis and organ differentiation. *Dev. Dyn.* **236**, 2899–2908

13. Sandell, L. L., Sanderson, B. W., Moisseyev, G., Johnson, T., Mushegian, A., Young, K., Rey, J. P., Ma, J. X., Staehling-Hampton, K., and Trainor, P. A. (2007) Rdh10 is essential for synthesis of embryonic retinoid acid and is required for limb, craniofacial, and organ development. *Genes Dev.* **21**, 1113–1124

14. Siegenthaler, J. A., Ashique, A. M., Zabalski, K., Patterson, K. P., Hecht, J. H., Kane, M. A., Fokas, A. E., Choe, Y., May, S. R., Kume, T., Napoli, J. L., Peterson, A. S., and Pleasure, S. J. (2009) Retinoic acid from the meninges regulates cortical neuron generation. *Cell* **139**, 597–609

15. Farjo, K. M., Moisseyev, G., Nikolaeva, O., Sandell, L. L., Trainor, P. A., and Ma, J. (2011) Rdh10 is the primary enzyme responsible for the first step of embryonic vitamin A metabolism and retinoid acid synthesis. *Dev. Biol.* **357**, 347–355

16. Ashique, A. M., May, S. R., Kane, M. A., Fokas, A. E., Phamluong, K., Choe, Y., Napoli, J. L., and Peterson, A. S. (2012) Morphological defects in a novel Rdh10 mutant that has reduced retinoid acid biosynthesis and signaling. *Genesis* **50**, 415–423

17. Lee, S. A., Belyaeva, O. V., Wu, L., and Kedischvili, N. Y. (2011) Retinol dehydrogenase 10 but not retinol/sterol dehydrogenase 17(s) regulates the expression of retinoic acid-responsive genes in human transgenic skin raft culture. *J. Biol. Chem.* **286**, 13550–13560
Rdh10 Localizes to Lipid Droplets

18. Boerman, M. H., and Napoli, J. L. (1996) Cellular retinol-binding protein-supported retinoic acid synthesis. Relative roles of microsomes and cytosol. J. Biol. Chem. 271, 5610–5616

19. Wang, J., Bongianni, I. K., and Napoli, J. L. (2001) The N-terminus of retinol dehydrogenase type 1 signals cytosolic orientation in the microsomal membrane. Biochemistry 40, 12533–12540

20. Belyaeva, O. V., Stetsenko, A. V., Nelson, P., and Kedishvili, N. Y. (2003) Properties of short-chain dehydrogenase/reductase RatR1. Characterization of purified enzyme, its orientation in the microsomal membrane, and distribution in human tissues and cell lines. Biochemistry 42, 14838–14845

21. Zhang, M., Hu, P., and Napoli, J. L. (2004) Elements in the N-terminal signaling sequence that determine cytosolic topology of short-chain dehydrogenases/reductases. Studies with retinol dehydrogenase type 1 and cis-retinol/androgen dehydrogenase type 1. J. Biol. Chem. 279, 51482–51489

22. Listenberger, L. L., and Brown, D. A. (2007) Fluorescent detection of lipid droplets and associated proteins. Curr. Protoc. Cell Biol. Chapter 24, Unit 24.2.2–24.2.11

23. Napoli, J. L., and Horst, R. L. (1998) Quantitative analyses of naturally occurring retinoids. Methods Mol. Biol. 89, 29–40

24. Kane, M. A., Folias, A. E., and Napoli, J. L. (2008) HPLC/UV quantitation of retinol, retinol, and retinyl esters in serum and tissues. Anal. Biochem. 378, 71–79

25. Bozidis, P., Williamson, C. D., and Colberg-Poley, A. M. (2007) Isolation of lipid droplets and associated proteins. Biochemistry 46, 290–329

26. Zhang, Y., Liu, C., Xiang, X., Jin, S., Zhang, P., Li, Q., Wang, D., Liu, X., Zeng, K., Zhang, J., Xiang, Y., and Zhang, C. Y. (2007) PGC-1α inhibits oleic acid induced proliferation and migration of rat vascular smooth muscle cells. PLoS ONE 2, e1137

27. Hess, D., Chisholm, J. W., and Igal, R. A. (2010) Inhibition of steraryl-CoA desaturase activity blocks cell cycle progression and induces programmed cell death in lung cancer cells. PLoS ONE 5, e11394

28. Magdalon, J., Hatanaka, E., Romanatto, T., Rodrigues, H. G., Kuwabara, W. M., Scaife, C., Newsholme, P., and Curi, R. (2011) A proteomic analysis of the functional effects of fatty acids in NIH 3T3 fibroblasts. Lipids Health Dis. 10, 218

29. Dickson, P. E., Tansey, I. T., and Welte, M. A. (2009) PAT proteins, an ancient family of lipid droplet proteins that regulate cellular lipid stores. Biochim. Biophys. Acta 1791, 419–440

30. MacDonald, P. N., and Ong, D. E. (1988) A lecithin:retinol acyltransferase activity in human and rat liver. Biochem. Biophys. Res. Commun. 156, 157–163

31. Batten, M. L., Imanishi, Y., Maeda, T., Tu, D. C., Moise, A. R., Bronson, D., Possin, D., Van Gelder, R. N., Baehr, W., and Palczewski, K. (2004) Lecithin:retinyl acyltransferase is essential for accumulation of all-trans-retinyl esters in the eye and in the liver. J. Biol. Chem. 279, 10422–10432

32. Liu, L., and Gudas, L. J. (2005) Disruption of the lecithin:retinyl acyltransferase gene makes mice more susceptible to vitamin A deficiency. J. Biol. Chem. 280, 40226–40234

33. Hayashi, T., Rizzuto, R., Hajnoczky, G., and Su, T. P. (2009) MAM. More than just a housekeeper. Trends Cell Biol. 19, 81–88

34. Rusiñol, A. E., Cui, Z., Chen, M. H., and Vance, J. E. (1994) A unique mitochondria-associated membrane fraction from rat liver has a high capacity for lipid synthesis and contains pre-Golgi secretory proteins including nascent lipoproteins. J. Biol. Chem. 269, 27494–27502

35. Stone, S. J., Levin, M. C., Zhou, P., Han, J., Walther, T. C., and Farese, R. V., Jr. (2009) The endoplasmic reticulum enzyme DGAT2 is found in mitochondria-associated membranes and has a mitochondrial targeting signal that promotes its association with mitochondria. J. Biol. Chem. 284, 5352–5361

36. Goodman, J. M. (2008) The gregarious lipid droplet. J. Biol. Chem. 283, 28005–28009

37. Listenberger, L. L., and Brown, D. A. (2008) Lipid droplets. Curr. Biol. 18, 2327–2338

38. Ducharme, N. A., and Bickel, P. E. (2008) Lipid droplets in lipogenesis and lipolysis. Endocrinology 149, 942–949

39. Fujimoto, T., and Parton, R. G. (2011) Not just fat: the structure and function of the lipid droplet. Cold Spring Harbor Perspect. Biol. 3, 10.1101/cshperspect.2142192

40. Brasaemle, D. L., Dolios, G., Shapiro, L., and Wang, R. (2004) Proteomic analysis of proteins associated with lipid droplets of basal and lipolytically stimulated 3T3-L1 adipocytes. J. Biol. Chem. 279, 46835–46842

41. Digel, M., Ehehalt, R., and Fülekrug, J. (2010) Lipid droplets lighting up. Insights from live microscopy. FEBS Lett. 584, 2168–2175

42. Bouchoux, J., Beilstein, F., Pauqui, T., Guerrera, I., C., Chateau, D., Ly, N., Alqub, M., Klein, C., Chambaz, J., Rocquet, M., Jacot, I., Morel, E., and Demignot, S. (2011) The proteome of cytosolic lipid droplets isolated from differentiated Caco-2/TC7 enterocytes reveals cell-specific characteristics. Biol. Cell 103, 499–517

43. Deisenroth, C., Itahana, Y., Tollini, L., Jin, A., and Zhang, Y. (2011) P53-inducible DHRS3 is an endoplasmic reticulum protein associated with lipid droplet accumulation. J. Biol. Chem. 286, 28343–28356

44. Haeseleer, F., Huang, J., Lebioda, L., Saari, J. C., and Palczewski, K. (1998) Molecular characterization of a novel short-chain dehydrogenase/reductase that reduces all-trans-retinal. J. Biol. Chem. 273, 21790–21799

45. Tajima, S., Goda, T., and Takase, S. (2001) Co-ordinated induction of an ancient family of lipid droplet proteins that regulate cellular lipid stores. Biochemistry 40, 6353–6368

46. Vichinsky, E., and Nigam, S. (2011) The proteome of cytosolic lipid droplets isolated from differentiated Caco-2/TC7 enterocytes reveals cell-specific characteristics. Biol. Cell 103, 499–517

47. Posch, K. C., Boerman, M. H., Burns, R. D., and Napoli, J. L. (1991) Holo- cellular retinol binding protein as a substrate for microsomal retinal synthesis. Biochemistry 30, 6224–6230

48. Ottonello, S., Scita, G., Mantovani, G., Cavazzini, D., and Rossi, G. L. (1993) Retinol binding to cellular retinol-binding protein is a substrate for cytosolic retinoid acid synthesis. J. Biol. Chem. 268, 27133–27142
59. Boerman, M. H., and Napoli, J. L. (1995) Characterization of a microsomal retinol dehydrogenase. A short-chain alcohol dehydrogenase with integral and peripheral membrane forms that interacts with holo-CRBP (type I). Biochemistry 34, 7027–7037

60. Malpeli, G., Stoppini, M., Zapponi, M. C., Folli, C., and Berni, R. (1995) Interactions with retinol and retinoids of bovine cellular retinol-binding protein. Eur. J. Biochem. 229, 486–493

61. Wolf, G. (1996) The regulation of retinoic acid formation. Nutr. Rev. 54, 182–184

62. Huang, D. Y., and Ichikawa, Y. (1997) Purification and characterization of a novel cytosolic NADP(H)-dependent retinol oxidoreductase from rabbit liver. Biochim. Biophys. Acta 1338, 47–59

63. Penzes, P., and Napoli, J. L. (1999) Holo-cellular retinol-binding protein. Distinction of ligand-binding affinity from efficiency as substrate in retinal biosynthesis. Biochemistry 38, 2088–2093

64. Napoli, J. L. (1999) Interactions of retinoid binding proteins and enzymes in retinoid metabolism. Biochim. Biophys. Acta 1440, 139–162

65. Jiang, W., and Napoli, J. L. (2012) Reorganization of cellular retinol-binding protein type 1 and lecithin:retinol acyltransferase during retinyl ester biosynthesis. Biochim. Biophys. Acta 1820, 859–869

66. Boerman, M. H., and Napoli, J. L. (1991) Cholate-independent retinyl ester hydrolysis. Stimulation by apo-cellular retinol-binding protein. J. Biol. Chem. 266, 22273–22278

67. Herr, F. M., and Ong, D. E. (1992) Differential interaction of lecithin-retinol acyltransferase with cellular retinol binding proteins. Biochemistry 31, 6748–6755

68. Rhinn, M., Schuhbaur, B., Niederreither, K., and Dollé, P. (2011) Involvement of retinol dehydrogenase 10 in embryonic patterning and rescue of its loss of function by maternal retinaldehyde treatment. Proc. Natl. Acad. Sci. U.S.A. 108, 16687–16692