thematic review

Thematic Review Series: Fat-Soluble Vitamins: Vitamin A

Enzymology of retinoic acid biosynthesis and degradation

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Abstract **All-*trans*-retinoic acid is a biologically active derivative of vitamin A that regulates numerous physiological processes. The concentration of retinoic acid in the cells is tightly regulated, but the exact mechanisms responsible for this regulation are not completely understood, largely because the enzymes involved in the biosynthesis of retinoic acid have not been fully defined. Recent studies using in vitro and in vivo models suggest that several members of the short-chain dehydrogenase/reductase superfamily of proteins are essential for retinoic acid biosynthesis and the maintenance of retinoic acid homeostasis. However, the exact roles of some of these recently identified enzymes are yet to be characterized. The properties of the known contributors to retinoid metabolism have now been better defined and allow for more detailed understanding of their interactions with retinoid-binding proteins and other retinoid enzymes. At the same time, further studies are needed to clarify the interactions between the cytoplasmic and membrane-bound proteins involved in the processing of hydrophobic retinoid metabolites. This review summarizes current knowledge about the roles of various biosynthetic and catabolic enzymes in the regulation of retinoic acid homeostasis and outlines the remaining questions in the field.—**Kedishvili, N. Y. **Enzymology of retinoic acid biosynthesis and degradation.** ***J. Lipid Res.*** **2013.** 54: **1744–1760.**

Supplementary key words vitamin A • retinol • dehydrogenase • reductase

All-*trans*-retinoic acid (atRA) is a highly potent derivative of vitamin A that is required for virtually all essential physiological processes and functions because of its involvement in transcriptional regulation of over 530 different genes (1, 2). For example, atRA is necessary for differentiation and development of fetal and adult tissues, stem cell differentiation, and apoptosis (3–7), and for support of reproductive functions (8, 9), immune response (10), and regulation of energy homeostasis (11, 12). atRA exerts its actions by serving as an activating ligand of nuclear atRA receptors [retinoid acid receptor (RAR)α, RARβ, and RARγ] and peroxisome proliferator-activated receptors (PPAR)β/δ, which form heterodimers with retinoid X receptors (13, 14). The actions of the RARs are described in more detail in this thematic series by Rochette-Egly and colleagues. The concentration of atRA during embryonic development is tightly controlled in a spatial and temporal manner, and in adult tissues, it is maintained within a very narrow range that is specific for each given tissue. If the control mechanisms fail and the concentration of atRA exceeds or falls below the optimal range, tissues and cells undergo pathophysiological changes that in most severe cases can lead to disease. Thus, the maintenance of optimal atRA levels is essential for life. This review focusses on recent findings regarding the mechanisms that control atRA homeostasis, with specific emphasis on the enzymes involved in atRA biosynthesis and degradation.

FROM DIETARY VITAMIN A TO CIRCULATING SERUM RETINOL

The precursors of atRA have to be obtained from the diet, either as retinyl esters from animal sources or provitamin A carotenoids from plants, mainly as β-carotene (reviewed in Refs. 15 and 16, and in this thematic series by Harrison and colleagues). The most recent advances in

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Abbreviations: atRA, all-*trans*-retinoic acid; 9cRA; 9-*cis*-retinoic acid; CRABPI, cellular retinoic acid-binding protein type I; CRBPI, cellular retinol-binding protein type I; CYP, cytochrome P450; RA, retinoic acid; RAL, retinaldehyde; RALDH1, retinaldehyde dehydrogenase 1; RAR, retinoic acid receptor; RBP4, serum retinol-binding protein 4; RDH, retinol dehydrogenase.

ADH, alcohol dehydrogenase; AKR, aldo-keto reductase; ALDH, aldehyde dehydrogenase; BCMO1, β-carotene monooxygenase type 1; ER, endoplasmic reticulum; LRAT, lecithin retinol acyl transferase; RL-HSD, retinol dehydrogenase-like hydroxysteroid dehydrogenase; RoDH4, retinol dehydrogenase 4; SDR, short-chain dehydrogenase/reductase; SR-B1, scavenger receptor class B, type 1.

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our understanding of postprandial processing of vitamin A are reviewed in details by other contributors to this series. Here, only the major steps are highlighted. Dietary retinyl esters are hydrolyzed in the intestine, and retinol taken into the enterocyte is reesterified. The dietary β-carotene absorbed in the small intestine is cleaved into two molecules of all-*trans*-retinaldehyde by β-carotene monooxygenase type 1 (BCMO1) (**Fig. 1**). Retinaldehyde is then reduced to all-*trans*-retinol by one or more retinaldehyde reductase(s) that are not yet well characterized. Retinol is esterified primarily by lecithin retinol acyltransferase (LRAT) and is incorporated into the lipid core of the chylomicrons, which are secreted into the lymphatic system. The retinyl esters associated with chylomicron remnants are cleared into hepatocytes where retinyl esters undergo a cycle of hydrolysis and reesterification before being deposited for storage in stellate cells. As needed, retinol is released from storage by retinyl ester hydrolases and is delivered to peripheral tissues in the form bound to plasma retinol-binding protein (RBP4) (16). This form of all-*trans*-retinol (holoRBP4) is the main source of vitamin A for most extrahepatic tissues. However, as discussed later in this article, some amount of atRA in target cells might be derived directly from circulating β-carotene.

BIOSYNTHESIS OF ATRA FROM RETINOL

atRA is produced from retinol in two oxidative steps: first, retinol is oxidized to retinaldehyde, and then retinaldehyde is oxidized to atRA. The first step, the oxidation of retinol to retinaldehyde, is generally considered to be rate-limiting (17), but as discussed later, in some cells, an increase in the rate of the second step appears to raise the steady-state levels of atRA (18, 19), suggesting that the second step may also be rate-limiting under certain conditions. Retinaldehyde can be converted back to retinol, but the oxidation of retinaldehyde to atRA is irreversible.

Reversible oxidation of retinol to retinaldehyde

Two types of enzymes have been implicated in the oxidation of retinol to retinaldehyde: the cytosolic alcohol dehydrogenases (ADH) of the medium-chain alcohol dehydrogenase family and the microsomal dehydrogenases of the short-chain dehydrogenase/reductase (SDR) superfamily of proteins (20). The current consensus appears to be that the ADH enzymes may contribute to the oxidation of retinol postnatally in specific tissues during vitamin A excess (ADH1) or deficiency (ADH4) but that they are not essential for atRA biosynthesis from a physiologically relevant supply of vitamin A during embryogenesis or adulthood (20–22). Thus, ADHs appear to play a role as backup enzymes under extreme dietary conditions.

The most recent studies focused on the role of SDR enzymes. SDRs represent one of the oldest and largest (over 46,000 members) protein superfamilies (23). Members of this superfamily contribute to essential metabolic functions in all forms of life. In humans, SDR enzymes are involved in numerous pathways, including metabolism of lipids, xenobiotics, prostaglandins, steroid hormones, and retinoids. The number of newly identified SDRs has grown so rapidly in the recent decade that it became essential to develop a systematic nomenclature for annotation and reference purposes (24). As a result, some of the retinoid-active SDRs previously known under multiple names received standardized names in accord with the new nomenclature and can now be easily identified and cross-referenced (**Table 1**). The new SDR nomenclature is gene based, and each member of every SDR family is given an individual designation. As a result, orthologous genes in different

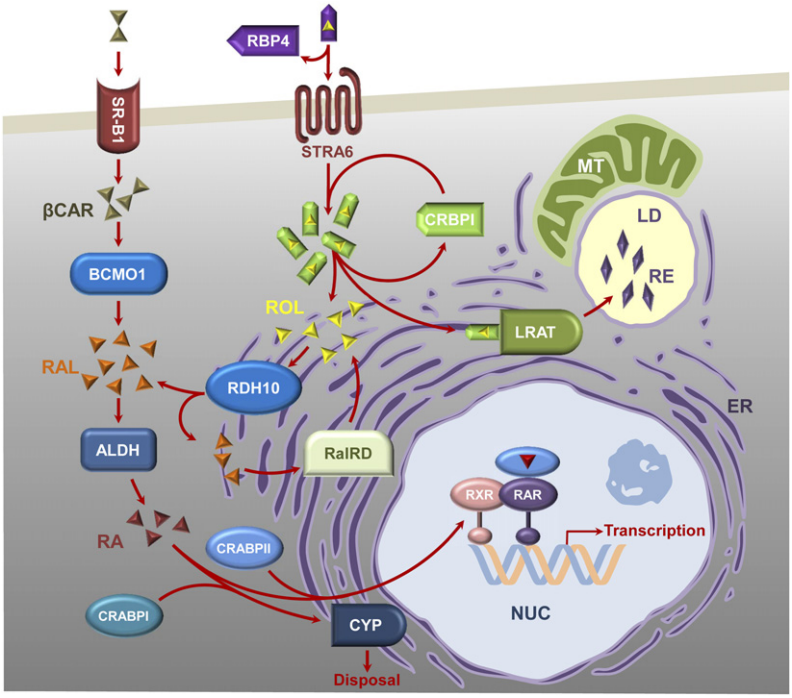

Fig. 1. Retinoic acid biosynthesis. Retinol (ROL, depicted as yellow pyramids) is delivered to extrahepatic cells bound to plasma RBP4. holoRBP4 binds to RBP4 receptor STRA6. CRBPI accepts retinol from STRA6 in the cytoplasm and delivers retinol to membranes, where retinol is either esterified by LRAT to REs (depicted as purple rhombuses) or oxidized by RDH10 to RAL (depicted as orange pyramids). RAL is oxidized further to RA (depicted as brown pyramids) by ALDH in the cytoplasm or is reduced back to retinol by retinaldehyde reductases (RalRD) in the membranes. RA binds to CRABP type I or type II and is transferred by holoCRABPII to the nucleus for binding to heterodimers of RAR and RXR or delivered to CYP enzymes by holoCRABPI for degradation. In addition, β-carotene (βCAR, depicted as duplicate of olive pyramids) is taken up by the cells through SR-B1 and cleaved into two molecules of retinaldehyde by BCMO1. Retinaldehyde derived from β-carotene may be oxidized to RA or converted to retinol as described above. LD, lipid droplet; MT, mitochondria; NUC, nucleus; RE, retinyl ester; RXR, retinoid X receptor.

TABLE 1. Nomenclature and properties of SDR enzymes

	Species	Cofactors[a]	Substrates	Nomenclature	Other Names
SDR7C					
RDH11	Human	NADP(H)	*at*ROL/RAL, *c*ROLs/RALs	SDR7C1	PSDR1, RalR1
	Mouse	NADP(H)	*at*ROL/RAL, *c*ROLs/RALs, nonanal, nonenal	SDR7C9	SCALD
RDH12	Human	NADP(H)	*at*ROL/RAL, *c*ROLs/RALs, nonanal, nonenal	SDR7C2	
	Mouse		ND	SDR7C10	
RDH13	Human	NADPH	*at*RAL	SDR7C3	
	Mouse		ND	SDR7C11	
RDH14	Human	NADP(H)	*at*RAL, 9*c*RAL, *at*ROL	SDR7C4	PAN2
	Mouse		ND	SDR7C12	
SDR9C					
RoDH4	Human	NAD(H)	*at*ROL/RAL, *c*ROLs/RALs, 3α-HS	SDR9C8	RDH16, RDH-E
RDH1	Mouse	NAD^+	*at*ROL, 9*c*ROL, 3α-HS	SDR9C17	
RoDH1	Rat	$NADP^+$, NAD(H)[b]	holoCRBPI, 3α-HS	SDR9C29	RDH7
RoDH2	Rat	$NADP^+$, NAD(H)[b]	holoCRBPI, 3α-HS	SDR9C28	RDH2
RL-HSD	Human	NAD(H)	*at*ROL, *at*RAL; 3α-HS	SDR9C6	HSD17B6, 3α-HSE
	Mouse	NAD(H)	*at*ROL/RAL, *c*ROLs/RALs; 3α-HS, 17β-HS	SDR9C13	HSD17B6, 17β-HSD9
DHRS9	Human	NAD(H)	3α-HS, may be *at*ROL	SDR9C4	RDHL, retSDR8, RDH-TBE, RoDH-E2, 3α-HSD
	Mouse		ND	SDR9C12	
	Rat		May be *at*ROL	SDR9C26	eRolDH2
11-*cis*-RDH	Human	NAD^+	*c*ROLs; 3α-HS	SDR9C5	RDH5
	Mouse	NAD(H)	*c*ROLs/RALs	SDR9C21	
HSD11B2	Human	NAD(H)	11β-HS	SDR9C3	Corticosteroid 11β-HSD
	Mouse			SDR9C11	
HSD17B2	Human	NAD(H)	17β-HS, 20α-HS	SDR9C2	Estradiol 17β-HSD
	Mouse			SDR9C10	
SDR16C					
retSDR1	Human	NADP(H)	*at*RAL	SDR16C1	DHRS3
	Mouse			SDR16C9	
RDH10	Human	NAD(H)	*at*ROL/RAL, *c*ROLs/RALs	SDR16C4	
	Mouse			SDR16C10	
RDHE2	Human	NAD(H)	*at*ROL	SDR16C5	
	Mouse	ND	*at*ROL	SDR16C11	
	Frog	NAD(H)	*at*ROL	SDR16C84	rdhe2, MGC80593
RDHE2S	Mouse	ND	*at*ROL	SDR16C12	
HSD17B11	Human	ND	3α-Androstanediol	SDR16C2	Pan1b, retSDR2
	Mouse			SDR16C7	
HSD17B13	Human	$NADP^+$	Cortisol	SDR16C3	SCDR10B
	Mouse			SDR16C8	

References to the studies in the table are cited in the text. Nomenclature for SDR proteins can be found at http://www.sdr-enzymes.org/. To search the database, choose field "family name" and enter SDR9C or SDR7C or SDR16C to view all currently known members. ND, no data.

[a] Some of the SDR enzymes can bind both NAD(H) and NADP(H), but their K_m values may differ by an order of magnitude. Only the preferred cofactors are listed.

[b] Rat RoDH1 and RoDH2 were reported to prefer $NADP^+$ with all-*trans*-retinol as substrate (90, 91) and NAD^+ with 3α-hydroxysteroids as substrates (182, 183).

species received their own unique identifiers; for example, human and mouse RDH10 genes are designated SDR16C4 and SDR16C10, respectively (Table 1). This is essential for tracking individual SDR members, as some human genes have multiple orthologs in other species (e.g., SDR9C family members) and as there is considerable confusion in the literature with multiple designations, aliases, names, and abbreviations. Such individual numbering of enzyme members has been recognized as a key to unambiguous referencing and has been successfully implemented for aldo-keto reductase (AKR) and cytochrome P450 (CYP) enzyme families.

In the past 17 years, three different families of SDRs have been implicated in the regulation of atRA homeostasis (**Fig. 2**). Interestingly, although the enzymes from different families share the ability to recognize retinol and retinaldehyde as substrates, their protein sequences are quite diverse (~30% overall sequence similarity). These significant differences in primary structures are reflected in the distinct profiles of substrate and cofactor specificities characteristic for each type of retinoid-active SDRs.

SDR9C family. Members of the SDR9C family of proteins were the first identified SDRs with retinol dehydrogenase activity (21). This group includes the 11-*cis*-retinol dehydrogenase (11cRDH, SDR9C5) (25), which was proven to be essential for the regeneration of 11-*cis*-retinaldehyde during the retinoid visual cycle, because mutations in this gene (RDH5) have been linked to fundus albipunctatus, a rare form of stationary night blindness characterized by a delay in the regeneration of cone and rod photopigments (26). Thus, at least one member of the SDR9C group is clearly important for retinoid metabolism in vivo. However, recombinant 11cRDH transiently expressed in

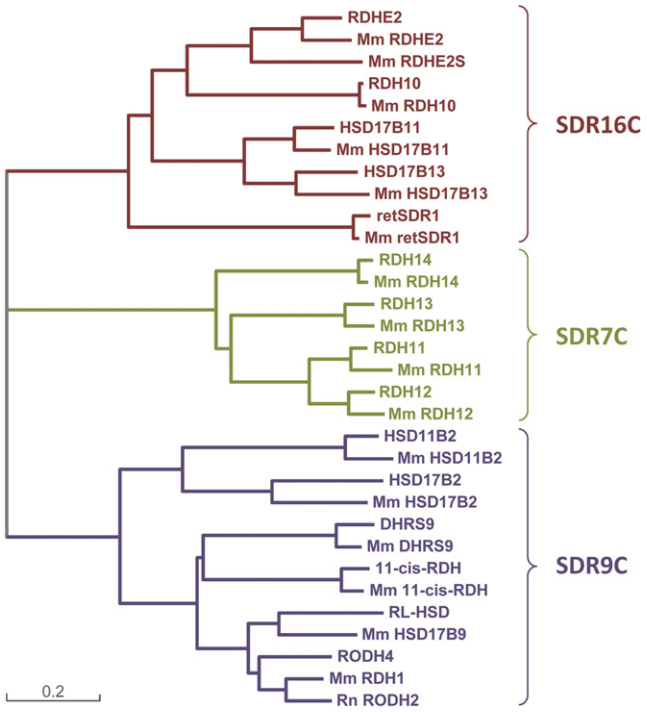

Fig. 2. Neighbor-joining phylogenetic tree of retinoid-active SDRs. Three branches of SDRs that include human and rodent retinoid-active enzymes implicated in retinoic acid biosynthesis are shown. SDR9C group is comprised of enzymes with preference for NAD(H) as cofactor. SDR7C group is composed of enzymes with preference for NADP(H) as cofactor. SDR16C group includes both NAD(H)- and NADP(H)-preferring enzymes. Murine enzymes are prefixed with Mm, and rat enzymes are prefixed with Rn. Scale bar, 0.2 amino acid substitutions per site.

COS cells or Chinese hamster ovary (CHO) K1 cells and analyzed using 100,000 *g* pellet or 830 *g* supernatant from lysed cells, respectively, had little or no activity toward all–*trans*-retinol (25, 27).

In humans, two enzymes of the SDR9C family show significant all-*trans*-retinol dehydrogenase activities in vitro and in cultured living cells (28–30). The first enzyme, retinol dehydrogenase 4 (RoDH4, SDR9C8 in Table 1), which was recombinantly produced in the microsomes of insect Sf9 cells using the Baculovirus expression system, exhibits wide retinoid isomer specificity, accepting both all-*trans*-retinol and *cis*-retinols as substrates (28). This enzyme has a very limited tissue distribution, being expressed primarily in the liver (liver>>>fetal lung>>brain). The second human enzyme with an all-*trans*-retinol dehydrogenase activity is retinol dehydrogenase-like hydroxysteroid dehydrogenase (RL-HSD, SDR9C6) (29). The latter enzyme is specific for all-*trans*-retinol and has a wider tissue distribution pattern, being most abundant in liver but also found in several extrahepatic tissues (liver>>>lung>placenta, brain, testis, prostate, spleen) (29).

It is important to point out that all members of the SDR9C family exhibit dual substrate specificity, recognizing not only retinoids but also 3α-hydroxysteroids (3α-androstane-diol, androsterone, allopregnanolone) as substrates (31). In fact, with the exception of 11cRDH, which shows similar activity toward 11-*cis*-retinol and 3α-androstanediol (27), all other SDR9C enzymes are several-fold more active toward 3α-hydroxysteroids than toward retinoids (reviewed in Ref. 20). For instance, androsterone added to cells stably transfected with RoDH4 or RL-HSD is fully metabolized within minutes, whereas the oxidation of all-*trans*-retinol takes several hours (30, 31). The K_m values of RoDH4 and RL-HSD for 3α-hydroxysteroids are comparable or even below those of purified recombinant NADPH-dependent cytosolic 3α-hydroxysteroid dehydrogenases of the AKR1 superfamily of proteins, which function in the reductive direction in the cells (32). In fact, recent evidence suggests that in prostate, the NAD^+-dependent RL-HSD works as a 3α-hydroxysteroid oxidase in tandem with the NADPH-dependent 3-ketosteroid reductase AKR1C2 to regulate the amount of 5α-dihydrotestosterone available for binding to the androgen receptor (33, 34). This observation brings up important questions. Can SDR9C enzymes contribute to both retinoid and steroid metabolism? Will the extent of their contribution depend on the cell-specific context? Answers to these questions are essential for defining the role of SDR9C enzymes in atRA biosynthesis.

Another important consideration is that atRA is essential for differentiation and development of all vertebrate species. Thus, it can be argued that atRA biosynthetic enzymes should be highly conserved across species because there is evolutionary pressure to preserve the functions of these enzymes. However, the properties of human SDR9C all-*trans*-retinol dehydrogenases are not well conserved in their mouse homologs. Unlike the human 11-*cis*-RDH, which shares 87% sequence identity with its mouse ortholog (SDR9C21), the mouse ortholog of RL-HSD (SDR9C13, 17β-HSD type 9, later renamed Hsd17b6) has 73% sequence identity with RL-HSD and exhibits a very low activity toward all-*trans*-retinol (reviewed in Ref. 35).

The second human SDR9C all-*trans*-retinol dehydrogenase, RoDH4, has not one but several orthologs in the mouse genome. Only one of these orthologs, RDH1 (SDR9C17), is active with all-*trans*-retinol as shown using the 800 *g* supernatant from RDH1-transfected CHO cells (36). Still, the catalytic efficiency of mouse RDH1 toward all-*trans*-retinol [V/K_m of 3 (nmol/min/mg)/μM] is ~10-fold lower than catalytic efficiency of RDH1 toward 3α-androstanediol [V/K_m of 31 (nmol/min/mg)/μM] (36). *Rdh1*-knockout mice are viable, indicating that *Rdh1* gene function is not essential for embryogenesis, but the adult mice have an interesting phenotype: instead of being smaller when restricted in vitamin A, they grow longer and larger than wild-type mice, with increased weight of multiple fat pads, liver, and kidney. There are no detectable changes in atRA levels in tissues of RDH1-null mice, possibly due to a downregulation (2.5-fold) of *Cyp26a1* expression, but the amount of retinol in mice on low vitamin A diet (0.6 IU/g) is increased in liver and kidney (1.5- to 2-fold) relative to wild-type (37). Thus, RDH1 clearly has an impact on retinoid metabolism in adult mice. Whether this is due to its retinol dehydrogenase activity and whether the contribution of RDH1 to atRA biosynthesis is the only role of RDH1 in mouse metabolism remains to be established.

Significance of RDH1 for steroid metabolism is difficult to evaluate in this mouse model, because the mouse genome contains multiple SDRs with 3α/17β-hydroxysteroid dehydrogenase activity (38). These redundant steroid dehydrogenases might compensate for the absence of functional RDH1 in steroid metabolic pathways.

A third member of the SDR9C family, DHRS9 (SDR9C4, also known as RDHL, 3α-HSD), has been implicated in atRA biosynthesis in humans (39). In our hands, human DHRS9 expressed in Sf9 cells and analyzed using DHRS9-enriched microsomal fraction has a negligible all-*trans*- or *cis*-retinol dehydrogenase activity in vitro (40), compared with similarly produced RoDH4 or RL-HSD (28, 29). However, others have reported that human DHRS9 and its rat ortholog (SDR9C26) contribute to atRA production when expressed in transiently transfected, living COS cells or astrocytes incubated with 2 μM retinol (19, 39). Surprisingly, silencing of rat DHRS9 expression in rat astrocytes leads to an increased rather than decreased conversion of retinol to atRA (19). The authors speculate that this increase in atRA biosynthesis is due to an upregulation in ALDH1A1 activity, which catalyzes the second step in atRA biosynthesis, the oxidation of retinaldehyde to atRA. An increase in ALDH1A1 activity would not be expected to accelerate atRA biosynthesis, since the oxidation of retinaldehyde to atRA is generally not considered to be the rate-limiting step of the pathway. Thus, it appears that this assumption does not apply to rat astrocytes. Regardless of whether DHRS9 acts as an all-*trans*-retinol dehydrogenase, this gene, in contrast to RoDH4 and RL-HSD, is quite conserved across species (86% identity between human and mouse), suggesting that DHRS9 must be physiologically important for survival. Further studies are justified and necessary to elucidate the molecular basis of its effect on atRA homeostasis.

Taken together, the current data suggest that SDR9C enzymes may play a role in the regulation of atRA homeostasis, either directly or indirectly, but that the contribution of individual SDR9C enzymes may be species-specific and cell context-dependent. The divergence in catalytic properties between mouse and human orthologs of RoDH4 and RL-HSD suggests that these enzymes have evolved to support specific metabolic needs of particular species but are not evolutionarily conserved in all vertebrates.

SDR16C family. The SDR16C family of SDRs has drawn a lot of attention recently because one of its members, mouse RDH10 (SDR16C10), was found to be essential for atRA biosynthesis during embryonic development (41). Human RDH10 was originally identified based on its sequence similarity to retina SDR1 (retSDR1, SDR16C1) (discussed below) (42, 43). The authors were searching for an enzyme that generates all-*trans*-retinaldehyde in the retinal pigment epithelium for the G protein-coupled receptor that isomerizes all-*trans*-retinaldehyde to 11-*cis*-retinaldehyde upon illumination. In vitro activity assays using 200 μg of microsomal fraction from COS cells transfected with RDH10/pCDNA6/V5/His expression construct and 1 μCi [^{3}H] all-*trans* retinol suggested that RDH10 acts as an NADP$^+$-preferring all-*trans*-retinol dehydrogenase (43). Under the same assay conditions, RDH10 did not oxidize 11-*cis*, 9-*cis*, or 13-*cis* retinol into the respective retinaldehydes in the presence of NAD$^+$ or NADP$^+$, leading the authors to conclude that RDH10 is specific for all-*trans*-retinol. The authors noted that RDH10 is highly conserved, with 99–100% amino acid sequence identities between bovine, mouse, and human RDH10, and they suggested that RDH10 may play a role in the generation of atRA. Others have reported that overexpression of human RDH10 in HepG2 cells induced a significant antiproliferative response and upregulation of RARβ (44), supporting the role of RDH10 in atRA biosynthesis. However, the ultimate proof came from recent mouse studies, which showed that inactivation of RDH10 function in mice either by mutations or a targeted gene knockout results in severe malformations and embryonic lethality due to insufficient production of atRA (41, 45, 46). This phenotype can be rescued by supplementation with atRA or all-*trans*-retinaldehyde (45, 46).

Biochemical analysis carried out in our laboratory revealed that human ortholog of RDH10 (SDR16C4) recognizes not only all-*trans*-retinol but also 11-*cis* and 9-*cis* retinols as substrates and strongly prefers NAD$^+$ as a cofactor, in agreement with the structural determinants of SDR cofactor specificity (47). This finding contradicted the previous report that human RDH10 has a significant activity with NADP$^+$ (43). The difference in results is most likely due to the distinct expression systems used to characterize human RDH10. Our studies were conducted using recombinant RDH10 expressed in microsomes of insect Sf9 cells, which have negligible background activity toward retinol, whereas the other group used an enzyme expressed in microsomes of mammalian COS cells. In our assays, 3–20 μg of microsomal protein was sufficient for a reliable detection of RDH10 activity with nonradiolabeled retinol. As discussed below, most mammalian cells have high endogenous levels of microsomal NADP(H)-dependent retinoid oxidoreductive activity that can work either in the oxidative or reductive direction when given the appropriate combination of substrates and cofactors (see the section on SDR7C enzymes). This endogenous NADP(H)-dependent activity might interfere with the analyses of substrate and cofactor specificities of the recombinantly expressed enzymes if NADP(H) is supplemented as a cofactor.

In comparison with SDR9C all-*trans*-retinol/sterol dehydrogenases, human RDH10 is not active toward 3α-hydroxysteroids, and it exhibits a much higher affinity for all-*trans*-retinol (~28-fold) than does either RoDH4 or RL-HSD (47). When overexpressed in a model of human organotypic skin raft culture, only RDH10 [not RoDH4, RL-HSD, or DHRS9 (RDHL)] induces a phenotype consistent with overproduction of atRA, which is characterized by an increased proliferation and reduced differentiation of keratinocytes (48). Thus, at least in human epidermis, SDR9C enzymes do not appear to contribute to atRA biosynthesis, whereas RDH10, a member of the SDR16C family, does.

RDH10 is the major retinol dehydrogenase responsible for atRA biosynthesis during embryonic development. However, the molecular patterning defects in RDH10-null mice do not reflect a complete state of atRA deficiency (45), suggesting that additional retinol dehydrogenases exist. RDH10 shares the highest sequence similarity with two other members of SDR16C family of proteins, RDHE2 (SDR16C5) and RDHE2S (SDR16C6) (Table 1 and Fig. 2). The genes encoding RDHE2 and RDHE2S are located in close proximity to RDH10 gene (SDR16C4) and may have originated from a common ancestor as a result of gene duplication. Proteins encoded by the mouse orthologs of human *RDHE2* and *RDHE2S* genes (SDR16C11 and SDR16C12, respectively) function as all-*trans*-retinol dehydrogenases in vitro and increase the rate of atRA biosynthesis from retinol when expressed in living cells (49, 50). Whether these two enzymes contribute to atRA biosynthesis during development or adulthood is not yet known, but studies in frogs indicate that the frog ortholog of these enzymes is essential for frog embryonic development (50). Frogs have a single gene, *rdhe2* (*sdr16c90*), in position orthologous to mouse *Rdhe2* (*Sdr16c11*) and *Rdhe2s* (*Sdr16c12*) genes. The enzyme encoded by the frog gene acts as a highly active retinol dehydrogenase that promotes atRA biosynthesis in living cells (50). Thus, the retinol dehydrogenase activity of RDHE2/RDHE2S enzymes is conserved in lower vertebrates. Further studies are needed to elucidate the roles of mammalian RDHE2 and RDHE2S in atRA biosynthesis. In addition to RDH10, RDHE2, and RDHE2S, this family includes two relatively weak steroid dehydrogenases, 17β-HSD11 (SDR16C2) (51) and 17β-HSD13 (SDR16C3) (52). Whether the latter two enzymes have activity toward retinoids has not been examined.

Reduction of retinaldehyde back to retinol

As pointed out above, the conversion of retinol to retinaldehyde is reversible. The direction catalyzed by the retinoid-active oxidoreductases is determined by the availability of the substrates and cofactors. If a given oxidoreductase has a similar affinity for both oxidized and reduced form of the same cofactor (e.g., NAD^+ versus NADH), then the cofactor binding site of such oxidoreductase in living cells will be occupied by the more prevalent form of the cofactor. It has been estimated that in liver cytosol, the concentration of the oxidized NAD^+ greatly exceeds that of NADH (1000:1), while the concentration of NADPH exceeds that of $NADP^+$ (1:100) (53). As a result, in intact cells, those oxidoreductases that have higher affinity for NAD(H) than for NADP(H) will preferentially bind the more abundant NAD^+ and, therefore, will function in the oxidative direction. On the other hand, the oxidoreductases that have higher affinity for NADP(H) will bind the more abundant NADPH and, therefore, will function in the reductive direction. Recent studies suggest that many types of cells contain NADP(H)-dependent retinoid oxidoreductases, which preferentially catalyze the reduction of all-*trans*-retinaldehyde to all-*trans*-retinol and compete for retinaldehyde with the retinaldehyde dehydrogenase that oxidizes all-*trans*-retinaldehyde to atRA. Considering that high levels of retinaldehyde can be toxic for cells and that retinaldehyde is the immediate precursor for atRA, it is not surprising that the levels of retinaldehyde are tightly controlled. Two types of enzymes have been proposed to catalyze the reduction of retinaldehyde back to retinol: the cytosolic AKRs and the microsomal SDRs.

The role of AKRs has been summarized in a recent review article (54). In brief, AKRs are enzymes with wide substrate specificity that catalyze the reduction of lipid peroxidation products (e.g., 4-hydroxy-*trans*-2-nonenal), ketosteroids, ketoprostaglandins, and xenobiotic compounds (reviewed in Ref. 55). The properties of AKR enzymes are not highly conserved across species. For example, AKR1B10 is the most potent all-*trans*-retinaldehyde reductase in humans, and AKR1B12, in chickens (54), but it is not clear whether this function is conserved in any of the rodent AKRs. This might indicate that AKRs are not involved in the maintenance of retinoid homeostasis under normal physiological conditions. However, human AKR1B10 is known to be overexpressed in certain types of cancers, including hepatocellular carcinoma and lung cancer associated with tobacco smoking (56–58). An upregulation of an enzyme with a potent retinaldehyde reductive activity, such as AKR1B10, might adversely affect the levels of atRA in cancer cells even though AKR1B10 may not be essential for the maintenance of atRA homeostasis under normal conditions.

retSDR1. RetSDR1 (SDR16C1, DHRS3) belongs to the same group of SDRs as RDH10, but it was reported to prefer NADPH as a cofactor and function as a retinaldehyde reductase (42). This conclusion was based on the experiments in which recombinant human retSDR1 expressed in the membrane fraction (40,000 rpm pellet) of Sf9 cells was shown to catalyze the transfer of ^3H from tritiated NADPH, but not NADH, to all-*trans*-retinal. Like RDH10, retSDR1 is highly conserved, with 98% sequence identity between human and mouse proteins. The transcript encoding retSDR1 is found in many human and rodent tissues (42), and the encoded protein is enriched at focal points of lipid droplet budding where it appears to promote lipid droplet storage (59, 60). Interestingly, the expression of retSDR1 in liver is sensitive to LPS-induced acute inflammation, suggesting that the reduction of retSDR1 levels in the liver may contribute to the perturbation of whole-body vitamin A metabolism that was shown to occur under inflammatory stress (61).

An observation that its expression is induced by atRA in human neuroblastoma cell lines (62) suggests that retSDR1 might act as an atRA-inducible gatekeeper to control the levels of retinaldehyde available for atRA biosynthesis. This hypothesis is supported by the recent in vivo studies in zebrafish, which show that manipulation of retSDR1 (dhrs3) expression levels in embryos leads to changes in expression levels of atRA-regulated genes (63). The role of retSDR1 in mammalian atRA homeostasis remains to be fully investigated.

SDR7C family. This family of SDR includes four enzymes with established in vitro retinaldehyde reductase activities: RDH11 (SDR7C1), RDH12 (SDR7C2), RDH13 (SDR7C3), and RDH14 (SDR7C4) (reviewed in Ref. 20). The founding member of this group, human RDH11 (SDR7C1, RalR1, PSDR1) exhibits an >800-fold higher affinity for NADP(H) than for NAD(H), and it is ~50-fold more efficient in the reductive direction than in the oxidative as determined using recombinant enzyme expressed in Sf9 cell microsomes. It recognizes all-*trans* and *cis*-retinoids with K_m values in the low micromolar range (K_m for retinaldehyde is ~0.12 μM), and it is not active toward steroid substrates (64, 65). Human RDH11 is anchored in the endoplasmic reticulum (ER) membrane by the N-terminal signal-anchor domain, with the majority of the polypeptide chain located on the cytosolic side of the membrane (65).

Although clearly a much better retinaldehyde reductase than a retinol dehydrogenase, in vitro, RDH11 exhibits a 10-fold greater rate for the oxidation of all-*trans*-retinol than RDH10 expressed at comparable protein levels in microsomes of Sf9 cells (Table 2 in Ref. 47). With its wide tissue distribution pattern, including all tested cell lines, RDH11 (or its orthologs in other species) will almost certainly contribute to the in vitro oxidation of all-*trans*-retinol by the cellular microsomal fractions if $NADP^+$ is added to the reaction mixtures as a cofactor. Therefore, it is likely that the $NADP^+$-dependent all-*trans*-retinol dehydrogenase activity of RDH11, and possibly of RDH14 (see below), may be the underlying cause of some discrepancies in reported substrate and cofactor specificities of the retinoid-active recombinant SDRs assayed using the membrane fractions of mammalian cells.

Human RDH11 has a single ortholog in mice, which shares 86% sequence identity with the human protein (66). Like the human enzyme, mouse RDH11 (SDR7C9) is active toward all-*trans*-retinaldehyde. In addition, the mouse enzyme can reduce short- and medium-chain lipid peroxidation aldehydes (e.g., nonanal, *cis*-6-nonenal, and *trans*-2-nonenal) to corresponding alcohols in vitro. However, this property is absent in the human enzyme, suggesting that the function of RDH11 in detoxification of lipid peroxidation products is not evolutionarily conserved. Interestingly, the expression level of mouse RDH11 in liver is regulated by sterol regulatory element binding proteins (SREBP), being suppressed by fasting and induced by refeeding, in accord with the changes in SREBPs (66). Like the human enzyme, mouse RDH11 mRNA is expressed in liver, retina, testis (in pachytene spermatocytes), adrenal gland, and ovary, but at lesser levels in prostate.

Rdh11 is expressed from day 7 of mouse embryonic development, but $rdh11^{-/-}$ mice do not exhibit any abnormalities in development, postnatal survival, or fertility (67). Nevertheless, RDH11 appears to have a measurable role in regenerating the visual pigment by complementing RDH5 as an 11*c*RDH in retinal pigment epithelium (RPE) cells. This finding implies that in RPE, RDH11 functions in the oxidative direction, which is possible because in the retina, the concentration of $NADP^+$ can exceed that of NAPDH by 1.5- to 4-fold (68). Unpublished data from our laboratory suggest that in extraocular tissues, RDH11 functions in the reductive direction and is essential for the maintenance of the cellular levels of all-*trans*-retinol during vitamin A deficiency (O. V. Belyaeva et al., unpublished data).

Located on human chromosome 14 next to RDH11 gene is the gene encoding RDH12, which shares 73% sequence identity with RDH11. Although closely related to RDH11, RDH12 exhibits somewhat different catalytic properties than RDH11. Similar to RDH11, RDH12 produced in the microsomes of Sf9 cells has a strong preference for NADP(H) as cofactor [2000-fold compared with NAD(H)], but unlike RDH11, RDH12 recognizes lipid peroxidation products as substrates in addition to all-*trans* and *cis* retinoids (69). Also, RDH12 is a much more active enzyme than RDH11 and remains the most catalytically efficient retinaldehyde reductase among the retinoid-active SDR and AKR enzymes known to date. When expressed in HEK293 cells, RDH12 functions exclusively in the reductive direction, increasing the amount of retinol and retinyl esters, but reducing the level of atRA (70).

Since its discovery, RDH12 has been the subject of great interest because mutations in RDH12 have been genetically linked to severe early-onset autosomal recessive retinal dystrophy (71–73). Human RDH12 is expressed almost exclusively in photoreceptor cells. Due to its specific expression in photoreceptors and retinaldehyde reductase activity, RDH12 was initially proposed to convert all-*trans*-retinaldehyde to all-*trans*-retinol in the recovery phase of the visual cycle (74). However, this hypothesis did not seem to be supported by the experimental evidence obtained using $Rdh12^{-/-}$ mice (74, 75), which had normal visual-cycle function. We suggested that, because RDH12 is localized in the inner segments rather than outer segments of photoreceptors, its primary function would be to protect the cells from excessive all-*trans*-retinaldehyde that diffuses into the inner segments from illuminated rhodopsin and can reach as high as 3 mM concentration (70, 76). As mentioned above, because of its ability to form Schiff bases with proteins and phospholipids, retinaldehyde can cause cytotoxicity by itself and also through excessive conversion to atRA. RDH12, with its exceptionally high catalytic efficiency, is ideally suited to handle large quantities of all-*trans*-retinaldehyde in photoreceptors. A recent study seems to support this hypothesis. Fluorescence imaging of single, isolated rod cells showed that, following exposure to light, outer segments appeared to leak all-*trans*-retinaldehyde to inner segments in rods lacking RDH12 (77).

Human RDH12 was reported to have some activity as a steroid reductase, reducing dihydrotestosterone to androstanediol when expressed in living HEK 293 T cells, but this activity is not conserved in its murine ortholog and is absent in other members of this group characterized to date (78).

RDH13 (SDR7C3) shares ~51% sequence identity with RDH11 and RDH12, and it is located on a different chromosome (chr. 19). Interestingly, unlike other members of this group, RDH13 is a mitochondrial rather than a microsomal protein, localized on the outer side of the

inner mitochondrial membrane (79). Another distinction is that RDH13 appears to exist as inactive dimer in the absence of dithiothreitol, but it converts to an active monomer upon addition of reducing agent dithiothreitol or glutathione. In this respect, RDH13 is similar to 11β-hydroxysteroid dehydrogenase type 2 of the SDR superfamily. It has been proposed that the inactive dimers could represent a latent form of the latter enzyme, and dimerization could serve as a mechanism for modulating the enzyme's activity (80). The activity of recombinant RDH13-His_6 purified from Sf9 cells using Ni^{2+}-affinity chromatography is also affected by the nature of the detergent and is sensitive to temperatures above ambient (79).

Kinetic analysis showed that purified RDH13 reduces all-*trans*-retinaldehyde (K_m of ~3 μM) with NADPH as the preferred cofactor, but it has little or no activity in the oxidative direction toward all-*trans*-retinol. Nonretinoid compounds, such as short- and medium-chain lipid aldehydes, derivatives of cholesterol, or acetoacetyl-CoA, do not inhibit the retinaldehyde reductase activity of RDH13, suggesting that they are not likely to be metabolized by RDH13 (79).

RDH13 is widely expressed in human tissues, but its expression levels vary considerably. It is relatively well conserved, sharing 83% protein sequence identity with mouse RDH13 and 72% identity with frog RDH13. The eyes of RDH13-null mice appear to be sensitive to intense light exposure (81), suggesting that RDH13 protects the retina against acute light-induced retinopathy. Furthermore, a recent study implicated RDH13 in pathogenesis of bilateral convergent strabismus with exophthalmus in German Brown cattle (82). Thus, RDH13 appears to be essential for the visual function, but further studies are necessary to evaluate the role of RDH13 in retinoid metabolism of extraocular tissues.

RDH14 (originally known as PAN2) shares ~40–46% sequence identity with RDH11 and RDH12. It is encoded by the gene located on chromosome 2, which appears to consist of only two exons. Human RDH14 polypeptide is longer (336 amino acids) than RDH11 (318 amino acids) and is relatively well conserved, sharing 84% identity with mouse RDH14 (SDR7C12). It is a ubiquitously expressed microsomal enzyme that exhibits a retinaldehyde reductase activity with high affinity for all-*trans*-retinaldehyde (K_m of ~0.08 μM) and NADPH (K_m of ~0.32 μM), but it can also function in the oxidative direction in vitro, albeit with a 4-fold lower efficiency as shown by assays using recombinant RDH14 produced in microsomes of Sf9 cells (83). RDH14 prefers all-*trans*-retinaldehyde over 9-*cis*-retinaldehyde and appears to be a more catalytically efficient all-*trans*-retinaldehyde reductase than RDH11 (83). Considering that it is expressed at high levels in at least 23 human tissues, RDH14 may be more essential for retinoid homeostasis than RDH11.

The redundancy of the SDR7C type of enzymes complicates the evaluation of their in vivo functions. Thus far, loss of function studies in RDH11 and RDH13 mice and the phenotypes caused by naturally occurring mutations in RDH12 and RDH13 suggest that these members of the SDR7C family of enzymes contribute to retinoid metabolism in the eye. As mentioned above, RDH11 also has a role in maintenance of retinol levels in extraocular tissues (O. V. Belyaeva et al., unpublished data). However, full evaluation of the in vivo contribution of the SDR7C family of enzymes to retinoid homeostasis may require generation of double ($Rdh11^{-/-}/Rdh14^{-/-}$) and potentially triple ($Rdh11^{-/-}/Rdh14^{-/-}/Rdh13^{-/-}$) knockouts of the respective genes.

Phylogenetic analysis suggests that the SDR7C group has deep evolutionary roots. At least six homologs of these genes sharing ~50% identity to human RDH12 can be found in the genome of *Drosophila melanogaster*. The proteins encoded by the *Drosophila* genes are 300–406 amino acids long and are associated with microsomal membranes (84). They recognize all-*trans*-retinaldehyde and all-*trans*-3-hydroxyretinaldehyde as substrates and prefer NADPH as a cofactor. *Drosophila* SDRs most likely contribute to the conversion of β-carotene and zeaxanthin to visual pigment since atRA signaling is absent in *Drosophila*, but phylogenetically, *Drosophila* SDRs belong to the same branch of the SDR superfamily as human RDH12, indicating a common ancestry early in bilaterian evolution before a protostome-deuterostome split.

Role of cellular retinol-binding proteins

Because all-*trans*-retinol is highly hydrophobic, it is found in cells either in cellular membranes or in the form bound to cellular retinol-binding proteins (CRBP) (85). Four types of CRBP have been reported in humans (86–88), but only three CRBPs exist in mice (86, 89). Mouse CRBPIII appears to be phylogenetically equivalent to human CRBPIV (88). CRBP type I exhibits the highest affinity for binding all-*trans*-retinol ($K_d \leq 3$ nM) and shows the broadest expression pattern in tissues. It has been proposed that CRBPI sequesters all-*trans*-retinol from nonspecific enzymes, such as ADHs (21). This hypothesis received support from studies that showed that all-*trans*-retinol bound to CRBPI (holoCRBPI) can be oxidized by purified rat liver RoDH1 (same as RoDH3, RDH7), the first SDR shown to have an all-*trans*-retinol dehydrogenase activity, but the reaction rate was 3- to 6-fold lower than that with free retinol (90, 91). This original observation suggested that holoCRBPI may directly bind to purified RoDH1, prompting us to search for an equivalent enzyme in humans. We were encouraged by our initial finding that the membrane-bound human RoDH4 expressed in microsomes of Sf9 cells produced retinaldehyde from holoCRBP1 (92). At the same time, this activity was not observed with similarly expressed RL-HSD, suggesting specific recognition of holoCRBPI by human RoDH4. However, subsequent analysis using a different HPLC mobile phase, which allowed a better separation of retinoid isomers, revealed that the actual product of RoDH4 activity toward holoCRBPI was 9-*cis*-retinaldehyde and not all-*trans*-retinaldehyde, because the chromatographically purified holoCRBP1 contained some amount of 9-*cis*-retinol, a proportion of which gradually increased with light exposure (69). An even faster accumulation of 9-*cis*-retinol was observed in the presence of human CRBPIII (E. V. Shabrova et al., unpublished observations), indicating that this phenomenon is not unique to CRBPI. Our finding provided a

reasonable explanation for the differential recognition of holoCRBPI by human RoDH4 versus human RL-HSD: RoDH4 oxidized the free 9-*cis*-retinol accumulated in holoCRBPI because 9-*cis*-retinol binds to CRBPI with much lower affinity than all-*trans*-retinol (93–95), whereas RL-HSD was inactive because, unlike RoDH4, it does not recognize 9-*cis*-retinol as substrate (29). To date, neither of the human SDRs characterized in our laboratory, including RDH10 and all of the SDR9C and SDR7C enzymes, was found to utilize CRBPI-bound all-*trans*-retinol in vitro. Others have come to a similar conclusion by showing that the addition of CRBPI to reactions containing microsomes with RDH10 inhibits rather than stimulates the oxidation of retinol (22).

Importantly, mice deficient in CRBPI have decreased levels of retinyl palmitate and retinol in liver, but their tissue levels of atRA are normal (96). Thus, CRBPI is most likely required for uptake and retention of retinol in the cells and, possibly, for chaperoning retinol between different types of cell membranes and/or lipid droplets (97, 98), but not specifically for atRA biosynthesis. An interesting study by Herr et al. (97) examined the ability of CRBPI and CRBPII to transfer retinol to phospholipid membranes by following the increase in intrinsic protein fluorescence that occurs upon ligand dissociation. The results showed that ligand transfer of retinol from CRBPI was >5-fold faster than transfer from CRBPII, and the rate of ligand transfer from CRBPI, but not from CRBPII, increased with increasing concentration of acceptor membrane and with the incorporation of the anionic lipid cardiolipin or phosphatidylserine into membranes. The authors concluded that transfer from CRBPI may involve and require effective collisional interactions with phospholipid membranes. Because transfer of retinol from CRBP to membranes does occur, it is possible that the retinol dehydrogenase obtains retinol directly from the phospholipid membranes. Alternatively, a collisional interaction of CRBPI with the retinol dehydrogenase could prompt the release of retinol from CRBPI and its transfer into the enzyme's active site. In this case, the transfer of retinol to phospholipid membranes containing RDH10 might occur at a higher rate compared with membranes that do not contain the enzyme. A crystallographic structure of RDH10 clarifying the entry point of the substrate into the active site relative to the membrane, similar to what has been done for prostaglandin endoperoxide H synthases-1 and 2 (99), might provide a definitive answer to this long-standing question.

It is worth noting that, in general, in vitro assays may not be adequate for analyzing the interaction between holoCRBP1 and retinol dehydrogenases because they do not reproduce the complex membrane environment that exists in living cells. More intact systems, such as whole-cell assays or mouse models, may provide a better insight into the interaction between holoCRBPI and the membrane-bound SDRs in their native environment. For example, a recent study revealed differences in subcellular localization of mouse RDH10 and mouse RDH1 in COS7 cells (100). RDH1 appeared to associate with the membranes of endoplasmic reticulum, whereas RDH10 colocalized with mitochondria/mitochondrial-associated membranes (MAM) in close proximity to CRBPI. During acyl ester biosynthesis, RDH10 partially relocated to lipid droplets. The authors reported that the specific activity of RDH10 associated with the lipid droplets was higher than that of RDH10 in MAM. However, the retinol dehydrogenase activity was measured in the presence of 10 mM $NADP^+$ as a cofactor. Because $NADP^+$ is the preferred cofactor for the SDR7C group of enzymes, the outcome of these in vitro measurements might have been affected by SDR7C enzymatic activities. Indeed, the authors noted a discrepancy between the low level of RDH10 expression in the ER and the disproportionately high level of retinol dehydrogenase activity.

Irreversible oxidation of retinaldehyde to retinoic acid

As suggested by numerous in vitro and in vivo studies, three members of the ALDH1A family of proteins are physiologically important for the oxidation of all-*trans*-retinaldehyde to atRA: ALDH1A1 (RALDH1), ALDH1A2 (RALDH2), and ALDH1A3 (RALDH3). In addition, another member of ALDH family, ALDH8A1 (RALDH4), was shown to recognize 9-*cis*-retinaldehyde but not all-*trans*-retinaldehyde as substrate (101–103). Interestingly, the amino acid sequence of RALDH4 is the least conserved among the ALDH1 group of enzymes (102).

The in vivo roles of individual ALDH isozymes in atRA biosynthesis have been summarized in several recent review articles (21, 104). In brief, ALDH1A2 is the primary enzyme responsible for atRA biosynthesis at most sites during embryogenesis. *Aldh1a2*$^{-/-}$ mice die early in embryonic development due to defects in heart morphogenesis (105). ALDH1A3 has a more limited role during development. *Aldh1a3*$^{-/-}$ mouse embryos survive until birth but die shortly thereafter from defects in nasal development (106). Mounting evidence suggests that ALDH1A3 plays a major role in protection of adult tissues against carcinogenesis (reviewed in Ref. 104). ALDH1A1 is not essential for embryogenesis, but it may have a role in atRA biosynthesis during adulthood. The in vivo role of ALDH1A1 in atRA biosynthesis is evidenced by the fact that, whereas *Aldh1a1*$^{-/-}$ mice are viable and have normal morphology of the retina, the livers of *Aldh1a1*$^{-/-}$ mice display reduced atRA biosynthesis and increased serum retinaldehyde levels after treatment with retinol (107, 108).

The primary goal of this review was to emphasize the differences in catalytic properties of the three major retinaldehyde dehydrogenases. Bhat and colleagues have performed extensive kinetic characterization of all three murine all-*trans*-retinaldehyde dehydrogenases under the same conditions, which allows for direct comparison of their properties (**Table 2**). ALDH1A3 is the most catalytically efficient enzyme, but it has a relatively high K_m value for all-*trans*-retinaldehyde (Table 2) (103). ALDH1A1 and ALDH1A2 have similar catalytic efficiency, but the K_m value of ALDH1A2 is much lower than that of ALDH1A1 (Table 2) (109, 110). Thus, ALDH1A1 is the least potent retinaldehyde dehydrogenase of the three enzymes. All three ALDHs recognize other substrates in addition to

TABLE 2. Kinetic properties of purified mouse retinaldehyde dehydrogenases

Parameters	ALDH1A1	ALDH1A2	ALDH1A3
K_m (μM)	11.6	0.66	3.9
V_{max} (nmol × min^{-1} × mg^{-1})	85.6	4.31	306

Mouse ALDH1A1 (RALDH1) and ALDH1A2 (RALDH2) proteins were expressed in *E. coli* as N-terminal fusions to glutathione-S-transferase (GST) and purified using GST-affinity column (109, 110). RALDH3 was expressed in *E. coli* as a His-tagged protein and affinity-purified using Ni-NTA column (103). The enzymatic products were analyzed by high pressure liquid chromatography.

retinaldehyde, including aldehydes derived from lipid peroxidation. In fact, ALDH1A1 plays a crucial role in protection of the mouse eye lens and cornea from lipid peroxidation aldehydes and cataract formation induced by oxidative stress, as demonstrated using various *Aldh1a1*$^{-/-}$ mouse models (111).

BIOSYNTHESIS OF ATRA FROM β-CAROTENE

Although most of the dietary β-carotene (60–70%) is absorbed and processed in the small intestine, the mRNA encoding β,β-carotene 15,15′-monooxygenase type 1 (BCMO1), which catalyzes the symmetrical cleavage of β-carotene into two molecules of all-*trans*-retinaldehyde (Fig. 1), is expressed in many other adult tissues and in mammalian embryo starting at seven days postconception (112–119). In mice, BCMO1 is detected at both mRNA and protein levels in liver, kidney, and testis in addition to the small intestine (112–116). In humans, BCMO1 is detectable by immunohistochemistry in the stomach, small intestine, colon, liver, kidney, skin, skeletal muscle, adrenal gland, pancreas, testis, ovary, prostate, endometrium, mammary tissue (117), and eyes (118, 119). This wide tissue distribution pattern suggests that BCMO1 can serve to provide the tissue-specific vitamin A supply in addition to retinol supplied by RBP4. Indeed, studies in BCMO1-knockout mouse models demonstrated that BCMO1 can maintain retinoid homeostasis in embryonic tissues of vitamin A-deficient mice (120–122). The all-*trans*-retinaldehyde produced by the cleavage of β-carotene can be directly oxidized to atRA by ALDH enzymes. However, this process is likely to be controlled through the competition between ALDH and retinaldehyde reductase enzymes, which compete for the same substrate, all-*trans*-retinaldehyde. β-carotene was shown to be converted to retinol in the liver (123), human colon cancer cells (124), human lung cells (125), skin fibroblasts (126), and prostate epithelial cell lines (127), confirming the presence of retinaldehyde reductases in these cells. Several interesting questions arise with respect to the enzymatic processing of β-carotene. First, both BCMO1 and aldehyde dehydrogenases are soluble proteins that act upon highly hydrophobic substrates. How do BCMO1 and ALDH get access to β-carotene and retinaldehyde? On the other hand, the SDR retinaldehyde reductases are integrated into the ER membranes. Do these enzymes retrieve retinaldehyde from the membranes, from cellular retinoid-binding proteins, or directly from BCMO1? What determines the rate of retinaldehyde conversion to retinol versus its conversion to atRA? Is it the relative level of retinaldehyde oxidizing versus reducing enzymatic activities, the effect of binding proteins, or a better access of certain enzymes to retinaldehyde? Answers to these questions are essential for our understanding of the enzymology of atRA biosynthesis and its regulation. Furthermore, while the molecular identities of β-carotene monooxygenase and retinoid-active aldehyde dehydrogenases have been established, the exact enzymes responsible for the conversion of retinaldehyde to retinol in the small intestine and various other tissues remain to be determined.

CATABOLISM OF ATRA

CYP26 family

Biochemical and genetic studies have identified three major enzymes, all of them members of the cytochrome P450 superfamily of proteins, which appear to be primarily responsible for the degradation of atRA (reviewed in Refs. 128 and 129). The enzymes that control atRA levels during embryonic development in mice are CYP26A1 and CYP26B1. CYP26A1-null mouse fetuses die at mid-late gestation, with multiple organ defects consistent with excessive atRA signaling (130). CYP26B1-null mice that are born alive die right after birth due to respiratory defects (131). During embryonic development, the two enzymes are expressed in distinct spatiotemporal patterns, with CYP26A1 being the major enzyme in human fetal brain and CYP26B1 found in all other tissues except brain. However, in adult human tissues, the expression patterns of CYP26A1 and CYP26B1 at the level of both mRNA and protein show a significant overlap (132). With the exception of liver, where CYP26A1 is the predominant form based on quantitative real-time PCR, and lung, where CYP26A1 is slightly more abundant, all other human adult tissues contain higher levels of CYP26B1 transcript. This tissue distribution pattern is supported by another study (133), with minor discrepancies that could be potentially attributed to variability in the quality of tissue samples.

Despite the low sequence identity (43%), CYP26A1 and CYP26B1 have very similar enzymatic properties, hydroxylating atRA to form 4OH-atRA and 18OH-atRA, and then hydroxylating these primary products further (132). Purified recombinant CYP26A1-His$_6$ has a lower affinity but a ~10-fold higher rate for formation of 4OH-atRA than does CYP26B1-His$_6$ (**Table 3**). CYP26A1 also has 2- to 10-fold

TABLE 3. Kinetic properties of purified human CYP26 enzymes

Parameters	CYP26A1	CYP26B1
K_m (nM)	50	19
V_{max} (pmol × min^{-1} × pmol P450^{-1})	10	0.8

CYP26A1 and CYP26B1 were expressed as C-terminally His_6-tagged proteins using the Baculovirus expression system and purified using a cobalt affinity column. The reaction products were analyzed by LC-MS/MS (132).

higher catalytic activity toward hydroxylated forms of atRA, making it ~20-fold more efficient than CYP26B1 in the overall depletion of atRA. Considering that CYP26A1 expression in liver is very sensitive to atRA levels (128), the high catalytic efficiency of this low-affinity enzyme would enable CYP26A1 to rapidly bring down the excessive levels of atRA.

The third cytochrome P450 enzyme implicated in catabolism of atRA is CYP26C1. This protein shares 45% amino acid identity with CYP26A1 and 51% with CYP26B1, but it exhibits a distinctly different expression pattern and catalytic properties than do CYP26A1 and CYP26B1. CYP26C1 is expressed mainly during embryonic development but appears to be nonessential, as CYP26C1-null mice have no apparent abnormalities (134). In adult tissues, it is detected at low levels in adrenal gland, lung, spleen, testis, brain, liver, and ovary (133). Unlike the other two enzymes, CYP26C1 prefers 9-*cis*-RA as substrate and exhibits broader substrate specificity in general. Furthermore, CYP26C1 shows a different response to treatment with atRA and 9-*cis*-RA, being upregulated in some tissues and cells but downregulated in others (129). Of the three CYP26 genes, CYP26C1 is the least conserved, and its physiological function remains poorly understood. A frameshift mutation in human CYP26C1 has been recently linked to focal facial dermal dysplasia type IV, a rare syndrome characterized by facial lesions resembling aplasia cutis (135).

In addition to the three CYP26 enzymes, several members of other CYP families, including human CYP3A4, CYP3A5, CYP3A7, CYP1A1, CYP4A11, CYP2C8, CYP2C9, CYP2C22, CYP2C39, CYP2S1, and CYP2E1 (136–150), have been shown to catabolize atRA. While CYP2C8 is catalytically more efficient than either CYP2C9 or CYP3A4, the overall contribution of CYP2C8 to atRA hydroxylation may not be much greater than that of CYP3A4 and CYP2C9, because the two latter enzymes are expressed at about 10-fold higher levels than CYP2C8 (139). Moreover, while the expression of both CYP2C8 and CYP2C9 genes is induced by atRA, CYP2C9 is significantly more responsive to atRA treatment (144), which would further increase its contribution to atRA catabolism.

Human CYP2S1 is expressed primarily in extrahepatic tissues, such as skin, trachea, lung, small intestine, kidney, breast, placenta, and colon (143). This gene was shown to be induced by atRA in skin but only in a subset of individuals. When expressed in *E. coli* together with NADPH cytochrome P450 reductase, CYP2S1 produced two metabolites of atRA that were identified as 4OH-atRA and 5,6epoxy-atRA (143). However, this activity was not observed with the N-terminally truncated CYP2S1 (145). The lack of activity in this case could be due to the alteration of the protein structure. Thus, the role of CYP2S1 in atRA hydroxylation remains to be fully investigated.

In rodents, rat CYP2C7, rat CYP2C22, and mouse CYP2C39 may contribute to atRA catabolism (136, 144, 146). The rat CYP2C7 is atRA 4-hydroxylase, which is induced by dietary retinoids in vitamin A-deficient mice (146, 147). The mouse homolog of this enzyme, CYP2C39, also exhibits atRA 4-hydroxylase activity (136). The rat CYP2C22, an ortholog of human CYP2C8 and CYP2C9, catabolizes atRA, and its expression is induced by vitamin A and exogenous RA through a canonical RA receptor-mediated mechanism, but the expression of CYP2C22 is essentially restricted to liver (144, 148). Interestingly, under normal dietary conditions, the relative level of CYP2C22 mRNA expression exceeds that of CYP26A1 by about 100-fold (148).

Mouse CYP2C39 has a relatively high affinity for atRA (K_m of 0.8 μM) but a ~40-fold lower V_{max} value compared with other CYP2C family members (136). Nevertheless, the decreased expression of this enzyme in the liver of mice lacking the aryl hydrocarbon receptor gene has been linked to increased levels of atRA, retinol, and retinyl palmitate (136).

CYP2E1 has long been known to catabolize atRA. Because chronic alcohol administration can lead to ~10-fold elevation of CYP2E1 activity, this enzyme may have a significant impact on atRA catabolism in alcoholic liver disease. In fact, ethanol was shown to enhance atRA metabolism into polar metabolites through the induction of CYP2E1 in the liver (149, 150).

Thus, kinetic characteristics and tissue distribution of various atRA-catabolizing enzymes indicate that while CYP26A1 and CYP26B1 may be the primary CYP enzymes for the hepatic clearance of atRA due to their high catalytic efficiency (141, 151), other members of CYP superfamily may play an essential role in extrahepatic tissues or in liver under certain conditions.

Role of cellular retinoic acid-binding proteins

Two types of cellular retinoic acid binding proteins (CRABP) are known to exist in vertebrates. CRABPI and CRABPII share approximately 75% amino acid homology (86), and both can bind atRA, 4oxo-atRA, 4OH-atRA, 9cRA, and 13cRA, but not retinol (152, 153). However, despite being quite similar, the two proteins appear to perform different cellular functions. CRABPI is thought to deliver atRA to CYP26 enzymes for degradation (154, 155), albeit to our knowledge, no studies documenting the direct protein-protein interaction between CYP26A1 or CYP26B1 and holoCRABPI have been published. The second type, CRABPII, is believed to channel atRA to the binding site of RARs (156). Both CRABPs are widely expressed in the embryo but their expression patterns do not usually overlap (157). In the adult, CRABPI is expressed almost ubiquitously, whereas CRABPII is often found in cells that synthesize high levels of atRA, such as skin (158), uterus, ovary (159, 160), and in the choroid plexus (161). Both CRABP isoforms are present in cytosol and nuclei of cells (162), although CRABPII is excluded

from the nuclei of spermatogonia (163), ovary (164), and uterus (165). Interestingly, in cortical cells of bovine adrenal, CRABPI is found associated with mitochondria (166). While the mechanism of CRABPII translocation to the nucleus has been addressed (167), it appears that less is known about the movement of CRABPI in and out of the nucleus and the functions of the two proteins in removing atRA from nuclei to terminate the signal.

The genes encoding CRABPI and CRABPII are highly conserved (86), suggesting an important physiological function, but laboratory mice lacking either or both of these genes are phenotypically normal, with the exception of a minor limb malformation. Moreover, *CrabpI*$^{-/-}$ / *CrabpII*$^{-/-}$ double-mutant embryos are not more sensitive than wild-type embryos to atRA excess treatment in utero (168). Nevertheless, a recent study shows that Crabps are essential for posterior patterning of the hindbrain in zebrafish (169), suggesting that other lipid-binding proteins might compensate for the absence of CRABPs in mice but not in zebrafish.

FEEDBACK REGULATION BY ATRA

As mentioned above, some of the components of the atRA biosynthetic and catabolic machinery are regulated by atRA via a feedback regulation loop. Excessive atRA coordinately induces the expression of the components of retinoid storage pathway, including CRBPI, LRAT (170–172), and retSDR1, which appear to promote the biosynthesis of retinyl esters (62). At the same time, atRA induces its own catabolism by upregulating CYP26 enzymes (173, 174), which degrade the excessive atRA (reviewed in Refs. 3 and 175). STRA6, the plasma membrane receptor for retinol carrier RBP4, is known to be induced by atRA in extrahepatic tissues (173, 174) while retinol-binding protein receptor 2 (RBPR2), the recently discovered analogous receptor in rodent liver, is suppressed by atRA, presumably to promote the uptake of retinol by extrahepatic tissues under conditions of vitamin A sufficiency (176).

The regulation of retinol dehydrogenases and retinaldehyde dehydrogenases by atRA is less well understood, and it appears that the responses of the genes encoding these enzymes may be species-specific and cell context-dependent. For example, vitamin A-deficient rats have lower levels of *Aldh1a1* (*RaldhI*) mRNA in kidney and liver but elevated levels of *Aldh1a1* in testis (177). Orally administered atRA restores *Aldh1a1* expression in kidney but not in liver (177). In primary keratinocytes, a high concentration of atRA (1 μM) appears to upregulate *ALDH1A3* (*RALDH3*), but it has no effect on *ALDH1A1* (*RALDH1*) or *ALDH1A2 (RALDH2)* expression (178). *RALDH3* expression is also induced by atRA in organotypic human skin cultures and in an epidermal explant, but it is not affected by atRA in dermal fibroblasts or HeLa cells. A somewhat more consistent pattern of atRA effect on *Aldh* genes is seen in mouse embryos, where treatment with atRA results in significant suppression of all three *Aldh* transcripts (179). However, chick ALDHs respond to neither excess nor deficiency of atRA (180).

The effect of atRA on the expression of RDH10 in human tissues and cell lines has not been fully investigated, but there seems to be a difference in the regulation of this gene among different species. For example, in *Xenopus laevis*, rdh10 expression is suppressed by atRA (181), but in chick, RDH10 is not affected by the excess or absence of atRA (180). A more systematic analysis of atRA effect on the expression of retinol dehydrogenases and retinaldehyde dehydrogenases is needed to better understand the effect of vitamin A status on the conversion of retinol to retinaldehyde in different species.

SUMMARY

The current state of knowledge suggests that there is a certain redundancy in the enzymes and proteins associated with each step in the conversion of retinol to retinaldehyde and further to atRA. RDH10 is the major retinol dehydrogenase during development, but other retinol dehydrogenases must exist and may play a more significant role in adulthood than during embryogenesis. In general, it appears that the lower-activity enzymes (ALDH1A1 and possibly RDHE2) may be sufficient for the maintenance of low levels of atRA in adulthood, whereas the more potent enzymes (RDH10, ALDH1A2, and ALDH1A3) are required for generation of high levels of atRA in spatially and temporally defined areas of embryo during development. RetSDR1 appears to be essential for the regulation of atRA homeostasis, but several members of the SDR7C group of enzymes also act as highly potent retinaldehyde reductases in vitro. The relative contribution of each of these enzymes to the reduction of retinaldehyde to retinol in vivo remains to be defined.

As with the biosynthesis of atRA, the degradation of atRA involves more than one enzyme. While the roles of CYP26A1 and CYP26B1 during development have been well defined, their overlapping expression patterns in adult tissues suggest that both are needed in the same tissue, perhaps to allow for a more flexible and sensitive adjustment of atRA levels in accord with specific physiological conditions. The presence of less-active CYPs from CYP3A and CYP2C families may allow for further fine-tuning of atRA levels. The in vivo activities of all CYP enzymes may depend on the levels of CRABPs and their ability to recognize CRABP-bound atRA as substrate. The exact mechanism of the interaction between CYP enzymes and CRABPs is another topic of interest that needs to be addressed.

Fundamental questions regarding the roles of cytosolic versus membrane-bound enzymes and shuttling of the hydrophobic substrates and products among them remain unanswered. For example, it is not clear how the retinaldehyde produced by the membrane-bound RDH10 is delivered to ALDHs, which are thought to be localized in the cytosol, since, like CRBPI, they partition with the soluble fraction during cell fractionation; and how the retinaldehyde produced by the cytosolic BCMO1 reaches the

membrane-bound SDR retinaldehyde reductases. On a purely intuitive level, it seems that all of the enzymes involved in retinoid metabolism should be in some way arranged around membranes, the most natural environment for lipophilic retinoids.

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