Eur. J. Biochem. **267**, 4315–4324 (2000) © FEBS 2000

MINIREVIEW

Families of retinoid dehydrogenases regulating vitamin A function

Production of visual pigment and retinoic acid

Gregg Duester

Burnham Institute, North Torrey Pines Road, La Jolla, CA, USA

Vitamin A (retinol) and provitamin A (β-carotene) are metabolized to specific retinoid derivatives which function in either vision or growth and development. The metabolite 11-*cis*-retinal functions in light absorption for vision in chordate and nonchordate animals, whereas all-*trans*-retinoic acid and 9-*cis*-retinoic acid function as ligands for nuclear retinoic acid receptors that regulate gene expression only in chordate animals. Investigation of retinoid metabolic pathways has resulted in the identification of numerous retinoid dehydrogenases that potentially contribute to metabolism of various retinoid isomers to produce active forms. These enzymes fall into three major families. Dehydrogenases catalyzing the reversible oxidation/reduction of retinol and retinal are members of either the alcohol dehydrogenase (ADH) or short-chain dehydrogenase/reductase (SDR) enzyme families, whereas dehydrogenases catalyzing the oxidation of retinal to retinoic acid are members of the aldehyde dehydrogenase (ALDH) family. Compilation of the known retinoid dehydrogenases indicates the existence of 17 nonorthologous forms: five ADHs, eight SDRs, and four ALDHs, eight of which are conserved in both mouse and human. Genetic studies indicate *in vivo* roles for two ADHs (ADH1 and ADH4), one SDR (RDH5), and two ALDHs (ALDH1 and RALDH2) all of which are conserved between humans and rodents. For several SDRs (RoDH1, RoDH4, CRAD1, and CRAD2) androgens rather than retinoids are the predominant substrates suggesting a function in androgen metabolism as well as retinoid metabolism.

Keywords: retinoid metabolism; retinoic acid; alcohol dehydrogenase; aldehyde dehydrogenase; short-chain dehydrogenase/reductase.

PHYSIOLOGICAL ROLES OF VITAMIN A

The function of vitamin A in higher animals is unique in that it functions in two completely different manners, i.e. light absorption for vision [1] and gene regulation for growth and development [2–4]. The functions of vitamin A are regulated by a battery of enzymes that control conversion of the alcohol form of vitamin A (retinol) to active retinoid aldehydes or carboxylic acids needed for either vision or growth and development. The metabolite needed for vision is the aldehyde isomer 11-*cis*-retinal, which functions as a light-absorbing pigment in the retina [1]. In order to regulate gene expression, retinol must be converted to the carboxylic acid isomers all-*trans*-retinoic acid or 9-*cis*-retinoic acid that serve as ligands for two families of retinoid receptors that directly regulate gene expression, i.e. the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) [5,6]. In most instances it appears that the active receptor is a RAR/RXR heterodimer that binds DNA regulatory sequences and regulates gene transcription in response to ligand binding [7]. RAR binds both all-*trans*-retinoic acid and its isomer 9-*cis*-retinoic acid, whereas RXR binds only 9-*cis*-retinoic acid suggesting unique functions for each family of receptors. Mice carrying knockout mutations of RARs or RXRs have multiple developmental defects, including those previously observed during vitamin A deficiency, thus providing proof that these receptors indeed mediate the functions of vitamin A during development [8–12].

The role of 11-*cis*-retinal in vision must be evolutionarily ancient as it used as the visual pigment by both invertebrate and vertebrate animals [13,14]. On the other hand, all-*trans*-retinoic acid and 9-*cis*-retinoic acid function as regulators of gene expression only in chordate animals, including all vertebrates as well as invertebrate chordates such as amphioxus and tunicates [15,16]. This chordate-specific function of vitamin A in gene regulation is mirrored by the evolution of RARs, which are present in all chordates examined, but absent in nonchordate animals such as arthropods (i.e. *Drosophila*) or nematodes [17,18]. *Drosophila* possesses a nuclear receptor called USP, which is most closely related to chordate RXR, but does not bind all-*trans*-retinoic acid or 9-*cis*-retinoic acid [19]. These findings indicate that the chordate animal lineage, the first to evolve retinoid receptors, should also possess unique vitamin A metabolic enzymes allowing production of all-*trans*-retinoic acid or 9-*cis*-retinoic acid to enable vitamin A to function in growth and development.

Studies in chordate animals are beginning to reveal a complex network of enzymes involved in the metabolism of vitamin A to active retinoid metabolites. Discussed below is evidence for the existence of three distinct families of retinoid

Correspondence to G. Duester, Gene Regulation Program, Burnham Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037, USA.
Fax: + 1 858 646 3195, Tel.: + 1 858 646 3138,
E-mail: duester@burnham.org

Abbreviations: ADH, alcohol dehydrogenase; Ahd, aldehyde dehydrogenase; ALDH, aldehyde dehydrogenase; CRAD, *cis*-retinol/androgen dehydrogenase; CRBPI, cellular retinol-binding protein type I; MDR, medium-chain dehydrogenase/reductase; RALDH, retinal dehydrogenase; RAR, retinoic acid receptor; RDH, retinol dehydrogenase; RoDH, retinol dehydrogenase; RXR, retinoid X receptor; SDR, short-chain dehydrogenase/reductase.

Enzymes: alcohol dehydrogenase (EC 1.1.1.1); aldehyde dehydrogenase (EC 1.2.1.3); short-chain dehydrogenase/reductase (EC 1.1.1.-).

(Received 28 February 2000, revised 25 April 2000, accepted 22 May 2000)

Table 1. Retinoid dehydrogenases of chordate animals.

Recommended name	Other names	Homologs identified in the following organisms
Alcohol dehydrogenase (ADH) family		
ADH1	Class I ADH, α, β, γ, *hADH1,2,3*	Mouse, rat, human, chick, frog, fish
ADH2	Class II ADH, π, ADHII, *hADH4*	Mouse, rat, human, ostrich
ADH4	Class IV ADH, σ, μ, *hADH7*, *mAdh7*, *mAdh3*	Mouse, rat, human, frog
ADH7	Class VII ADH, ADH-F	Chick
ADH8	Class VIII ADH, NADP(H)-dependent ADH	Frog
Short-chain dehydrogenase/reductase (SDR) family		
RoDH1	RoDH(I)	Mouse, rat
RoDH2	RoDH(II)	Mouse, rat
RoDH3	RoDH(III)	Rat
RoDH4	hRDH-E	Human
CRAD1		Mouse
CRAD2		Mouse
RDH5	11-*cis*-RDH, 11-*cis*-RoDH, RDH4, 9cRDH	Mouse, human, bovine
retSDR1		Mouse, human, bovine
Aldehyde dehydrogenase (ALDH) family:		
ALDH1	Class I ALDH, Ahd2, RALDH, RalDH(I)	Mouse, rat, human, chick, frog
ALDH6	V1 enzyme	Mouse, human, chick
RALDH2	RALDH-2, RalDH(II), V2 enzyme	Mouse, rat, human, chick
ALDH-t		Tunicate

dehydrogenases that participate in the interconversion of various isomers of retinol, retinal, and retinoic acid to produce the active retinoids needed for either vision or gene regulation. These enzymes are members of three families each having an ancient origin, i.e. alcohol dehydrogenase (ADH) [20,21], short-chain dehydrogenase/reductase (SDR) [22], and aldehyde dehydrogenase (ALDH) [23]. ADHs are members of the medium-chain dehydrogenase/reductase (MDR) family, which for the most part consists of enzymes containing subunits of about 350 residues with active sites possessing catalytic a zinc [24]. ADHs have wide substrate specificities and are responsible for the metabolism of ethanol, retinoids, and many other alcohols and aldehydes of physiological importance [21]. SDR enzymes typically have subunits containing approximately 250 residues (thus shorter than ADHs or MDRs), with active sites including the motif YXXXK but lacking a metal ion [22]. SDRs, like ADHs, have wide substrate specificities encompassing many physiologically important alcohols and aldehydes including hydroxysteroids and retinoids [25]. ALDH enzymes typically have subunits about 500 residues

Table 2. Functions of retinoid dehydrogenases.

Enzyme	Cofactor pref.	Preferred substrate activity
Alcohol dehydrogenase (ADH) family		
ADH1	NAD	Oxidation: all-*trans*-retinol and 9-*cis*-retinol
ADH2	NAD	Oxidation: all-*trans*-retinol and 9-*cis*-retinol
ADH4	NAD	Oxidation: all-*trans*-retinol and 9-*cis*-retinol
ADH7	NAD	Oxidation: all-*trans*-retinol (equally active with androgens)
ADH8	NADPH	Reduction: all-*trans*-retinal and 9-*cis*-retinal
Short-chain dehydrogenase/reductase (SDR) family		
RoDH1	NADP	Oxidation: all-*trans*-retinol (androgen activity 25-fold higher)
RoDH2	NADPH	Reduction: all-*trans*-retinal (stimulated when bound to CYP2D1)
RoDH3		Probably same as RoDH1 which shares 98% sequence identity
RoDH4	NAD	Oxidation: all-*trans*-retinol (androgen activity 15-fold higher)
CRAD1	NAD	Oxidation: 9-*cis* + 11-*cis*-retinol (androgen activity 60-fold higher)
CRAD2	NAD	Oxidation: 11-*cis*-retinol (androgen activity 40-fold higher)
RDH5	NAD	Oxidation: 11-*cis*-retinol (visual cycle) and 9-*cis*-retinol
retSDR1	NADPH	Reduction: all-*trans*-retinal (visual cycle)
Aldehyde dehydrogenase (ALDH) family		
ALDH1	NAD	Oxidation: all-*trans*-retinal and 9-*cis*-retinal
ALDH6	NAD	Oxidation: all-*trans*-retinal
RALDH2	NAD	Oxidation: all-*trans*-retinal
ALDH-t	NAD	Oxidation: all-*trans*-retinal

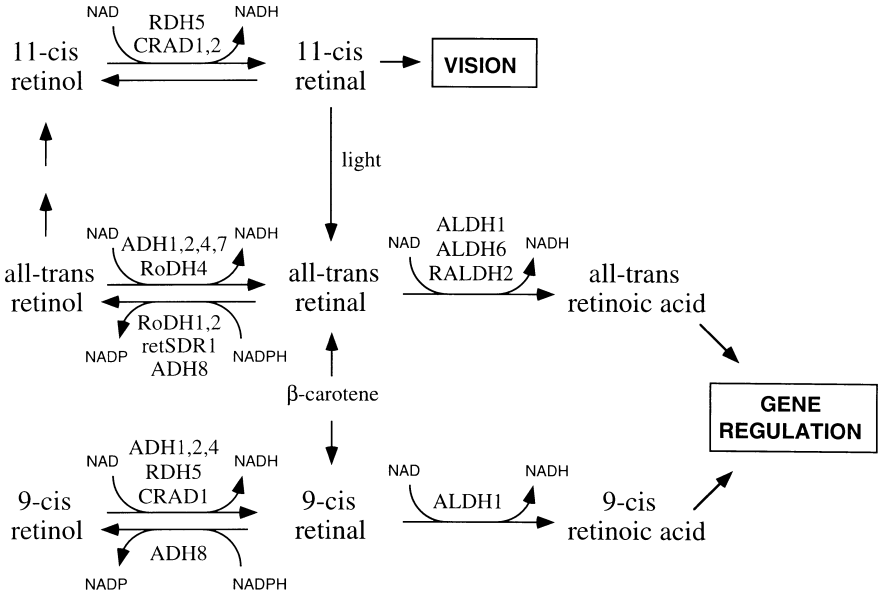

Fig. 1. Metabolic steps catalyzed by retinoid dehydrogenases during vitamin A metabolism. The ability to reversibly oxidize/reduce retinol and retinal isomers has been demonstrated for several members of the ADH family (ADH1, ADH2, ADH4, ADH7, ADH8) and several members of the SDR family (RoDH1, RoDH2, RoDH4, CRAD1, CRAD2, RDH5, retSDR1). ADHs and SDRs preferring NAD(H) as cofactor are shown catalyzing alcohol oxidation, whereas those preferring NADP(H) as cofactor are shown catalyzing aldehyde reduction. The irreversible oxidation of retinal isomers to retinoic acid isomers has been demonstrated for several members of the ALDH family (ALDH1, ALDH6, RALDH2) all of which use NAD as cofactor.

in length with activesites containing a catalytic cysteine [23]. ALDHs alsohave a wide substrate specificity, metabolizing acetaldehyde, retinoids, and many other physiologically important aldehydes to the corresponding carboxylic acids [26]

Those ADHs, SDRs, and ALDHs that have been implicated as retinoid dehydrogenases are examined further here. A listing of known chordate retinoid dehydrogenases representing non-orthologous forms is presented, which provides for each enzyme a single unified name to represent all orthologs identified across species boundaries (Table 1). The vertebrate ADH nomenclature which has been previously reported includes the names ADH1–ADH7 that are used here [21]. Recently, a new class of ADH distinct from ADH1–7 has been described in the frog [27], and this enzyme has been given the name ADH8 here. The SDRs involved in retinoid metabolism have been previously reviewed [28], and the names described therein have been used here with the exception of RDH4 which is now known to be the mouse homolog of human RDH5 (see below). The long-standing nomenclature for human ALDHs (ALDH1–10) which includes ALDH1 and ALDH6 [29] is used here for those enzymes; RALDH2 represents a new form never formally included in that nomenclature and ALDH-t is a new name proposed first here. A recently described new ALDH nomenclature based upon that used for P450 enzymes would place all the ALDHs described in Table 1 within the same subfamily ALDH1A [30]. The mouse has been examined most extensively and has so far been found to possess 12 of the 17 enzymes listed here. Of these 12 enzymes, eight are also found in human (ADH1, ADH2, ADH4, RDH5, retSDR1, ALDH1, ALDH6, and RALDH2) suggesting they have conserved functions.

The cofactor and substrate preferences for each retinoid dehydrogenase are summarized (Table 2). It is noted that the SDR enzymes RoDH1, RoDH4, CRAD1, and CRAD2 have much higher activities with androgen substrates than with retinoid substrates (Table 2), suggesting that the *in vivo* roles of these enzymes may lie primarily in the control of hydroxysteroid metabolism. In general, retinoid dehydrogenases preferring NAD(H) are most likely to perform oxidations *in vivo* and those preferring NADP(H) are most likely to perform reductions *in vivo* as previously reviewed [31] and as shown in Fig. 1.

ALCOHOL DEHYDROGENASE (ADH) FAMILY

ADH1

Liver ADH1 was one of the first retinoid dehydrogenases to be identified, and was found to function *in vitro* as a catalyst for the NAD-dependent oxidation of retinol to retinal [32,33]. It is now known that there exists a family of ADHs able to utilize retinoids, all of which are cytosolic zinc-dependent enzymes with subunit molecular masses around 40 kDa [31]. Humans possess three isoforms of ADH1 (ADH1A, B, and C sharing about 94% sequence identity), whereas rodents possess one form [21]. Purified rat ADH1 and human ADH1A have been demonstrated to catalyze the oxidation of all-*trans*-retinol similarly ($k_{cat}/K_m = 0.3\ \mu M^{-1}\cdot min^{-1}$), whereas the other human isoforms have less activity [34–36]. Thus, ADH1 may contribute to the production of all-*trans*-retinal that is further metabolized to all-*trans*-retinoic acid needed for gene regulation. Because alcohol dehydrogenation is a reversible reaction, ADH1 can also catalyze the NADH-dependent reduction of retinal to retinol [34,35]. However, under physiological conditions where the ratio of NAD/NADH is close to 1000 [37], ADH1 can contribute significantly only to retinol oxidation.

ADH1 has also been demonstrated to utilize 9-*cis*-retinoids, but the relative catalytic efficiencies for oxidation of all-*trans*-retinol and 9-*cis*-retinol have not been reported [34,35,38]. X-ray crystal structures of ADH1 from horse, human, and cod fish reveal a conserved active site that can accommodate long-chain alcohols [39–41]. Molecular docking studies have shown that the ADH1 active site is well-designed to bind all-*trans*-retinol [42], but that 9-*cis*-retinol does not fit as well as all-*trans*-retinol [43]. The human ADH1B isoform has been found to utilize 3α,5β-hydroxysteroids (bile acid intermediates) [44]. Human ADH1C, but not ADH1A or ADH1B, can metabolize 3β,5β-hydroxysteroid substrates but not 5α-hydroxysteroids [45]. Because steroid hormones have the 5α configuration when possessing a hydrogen at that position, whereas bile acids have the 5β configuration, ADH1B and ADH1C may contribute to bile acid metabolism rather than steroid hormone metabolism. On the other hand, ADH1A does not metabolize hydroxysteroids, but is efficient with all-*trans*-retinol as mentioned above.

ADH1 is conserved across many species including bony fish, frogs, birds, rodents, and humans; amino-acid sequence conservation between mouse and human forms is 85% [21]. ADH1 is found at very high levels in liver representing about 1% of cytosolic liver protein in humans [46], mouse [47], frog [48], or cod fish [49]. However, ADH1 is also expressed at high levels in many retinoid target tissues including human fetuses [50], mouse embryos [51,52], frog embryos [53], adult mouse (intestine, kidney, adrenal, testis, epididymis, uterus, ovary) [54–57] and adult frog (intestine, mesonephros, air sac) [53]. Thus, the expression pattern of ADH1 is indicative of a role in retinoid signaling during development and then afterwards in several adult epithelial tissues known to require retinoic acid for proper differentiation.

The ADH inhibitor 4-methylpyrazole has been shown to inhibit metabolism of a dose of all-*trans*-retinol in mice resulting in lower levels of all-*trans*-retinoic acid in embryos and maternal plasma [58]. However, it was not clear if the inhibitory effect was on ADH1 or another ADH. Genetic studies have shown that *Adh1* knockout mice lacking ADH1 activity exhibit a large reduction in the metabolism of a dose of all-*trans*-retinol to all-*trans*-retinoic acid compared to wild-type mice [59]. This provides evidence that ADH1 indeed functions as a retinoid dehydrogenase *in vivo*. However, survival of *Adh1* knockout mice under normal laboratory conditions indicates that at least one additional retinol dehydrogenase exists that can produce sufficient all-*trans*-retinal to fuel synthesis of all-*trans*-retinoic acid and thus compensate for a lack of ADH1.

ADH2

ADH2 is a distinct class of cytosolic ADH sharing 60% sequence identity with ADH1 [21]. Human ADH2 been demonstrated to catalyze NAD-dependent oxidation of all-*trans*-retinol [35,36] and may thus contribute to the production of all-*trans*-retinal needed for retinoic acid synthesis. Rat ADH2 is more active with 9-*cis*-retinol ($k_{cat}/K_m = 0.47\ \mu M^{-1}\cdot min^{-1}$) and has relatively low activity with all-*trans*-retinol ($k_{cat}/K_m = 0.09\ \mu M^{-1}\cdot min^{-1}$), but it accounts for only 2% of the cytosolic retinol dehydrogenase activity in rat liver whereas ADH1 accounts for $> 80\%$ [38]. In contrast to these studies, mouse ADH2 has no activity with all-*trans*-retinol (9-*cis*-retinol activity was not tested) [60]. Mouse and human forms of ADH2 have relatively low sequence conservation (72%) and many differences in substrate specificity, but both have high activity with benzyl alcohol and aldehyde substrates [60] suggesting that ADH2 may have evolved for this purpose rather than for retinoid metabolism. In addition, ADH2 is inactive with hydroxysteroid substrates [45]. ADH2 has a very limited tissue distribution, being found only in adult liver [60] and possibly human skin [61]. These findings suggest that ADH2 may not play an evolutionarily conserved role as a retinol dehydrogenase, but may play a species-specific role.

ADH4

ADH4 represents another class of cytosolic ADH sharing 70% sequence identity with ADH1 [21]. Mouse, rat, and human ADH4 have been shown to quite efficiently catalyze NAD-dependent oxidation of either all-*trans*-retinol [34–36,62,63] or 9-*cis*-retinol [64] and may thus contribute to the production of all-*trans*-retinal and 9-*cis*-retinal for synthesis of all-*trans*-retinoic acid and 9-*cis*-retinoic acid. A comparison of human ADHs for oxidation of all-*trans*-retinol has demonstrated that ADH4 is most efficient. Catalytic efficiencies are as follows: ADH1 isoforms ($k_{cat}/K_m = 0.01–0.3\ \mu M^{-1}\cdot min^{-1}$), ADH2 ($k_{cat}/K_m = 0.5–0.7\ \mu M^{-1}\cdot min^{-1}$), and ADH4 ($k_{cat}/K_m = 1.9–4.5\ \mu M^{-1}\cdot min^{-1}$) [35,36]. A comparison of the catalytic efficiencies of human ADH4 for all-*trans*-retinol ($k_{cat}/K_m = 4.5\ \mu M^{-1}\cdot min^{-1}$) and 9-*cis*-retinol ($k_{cat}/K_m = 13.3\ \mu M^{-1}\cdot min^{-1}$) indicates that the enzyme prefers the *cis* substrate [64], thus contrasting with ADH1 which prefers the *trans* substrate [35]. The tertiary structure of ADH4 has shown that it, like ADH1, has an active site well-suited to bind long-chain alcohols such as all-*trans*-retinol [65]. Substrate docking studies have indicated that all-*trans*-retinol, 9-*cis*-retinol, and 11-*cis*-retinol can bind better to the ADH4 active site than to the ADH1 active site [42,43]. ADH4 is inactive with hydroxysteroid substrates [66].

Rodent and human ADH4 orthologs are highly conserved, sharing 90% sequence identity [21]. A frog cDNA has been found to most likely encode ADH4 based upon its sequence homology to mammalian forms (65%) and its highly conserved pattern of expression that is highly conserved with that of the mammalian forms [53]. ADH4 is not expressed in the liver, but is found at high levels in the epithelial cells of the stomach, esophagus, skin, eye, adrenal, and reproductive tissues of mouse [55–57,67] and stomach, esophagus, and skin of frog [53] as well as in mouse embryos [51,52] and in several ocular tissues where it may play a role in the visual cycle [68]. High expression in many retinoid target tissues suggests a role as a retinoid dehydrogenase.

Genetic studies on *Adh4* knockout mice have demonstrated that a lack of ADH4 leads to decreased fetal survival during vitamin A deficiency compared to wild-type mice [69]. This provides physiological evidence that ADH4 retinol dehydrogenase activity indeed functions in retinoic acid synthesis during development. However, ADH4 may only be important when vitamin A is low in the diet. The survival of *Adh4* knockout mice under normal laboratory conditions (i.e. vitamin A sufficiency) shows that another retinol dehydrogenase can compensate for the lack of ADH4 under these conditions.

ADH7

An ADH identified in the fetal chick originally called ADH-F was found to catalyze NAD-dependent oxidation of all-*trans*-retinol to produce all-*trans*-retinal [70]. ADH-F was found to be a unique form of cytosolic ADH sharing 67% sequence identity with ADH1 and was thus given the name class VII ADH or ADH7 [21]. No ADH7 homolog has been identified in mammals or any other chordate animal and its expression appears to be quite limited, so far being detected only in liver [70]. ADH7 is equally active in the oxidation of either all-*trans*-retinol ($k_{cat}/K_m = 0.03\ \mu M^{-1}\cdot min^{-1}$) or various 3β,5α-hydroxysteroids ($k_{cat}/K_m = 0.02\ \mu M^{-1}\cdot min^{-1}$), but is inactive with 3β,5β-hydroxysteroids [70], suggesting that this enzyme may also metabolize specific steroids. More studies are needed to determine if ADH7 plays an important role as a retinoid or hydroxysteroid dehydrogenase.

ADH8

A recently discovered form of cytosolic ADH has been identified in the frog *Rana perezi* that prefers NADP(H) over NAD(H) and shares about 60% sequence identity with ADH1 [27]. This represents the only form of vertebrate ADH known that prefers the phosphorylated cofactor, and it is thus recommended that this form be given a new designation as class VIII

ADH or ADH8. ADH8 has not yet been reported in a mammalian species. ADH8 should be expected to catalyze reduction reactions *in vivo* due to the high ratio of NADPH to NADP physiologically [37]. Indeed, ADH8 has very high activity for reduction of all-*trans*-retinal to all-*trans*-retinol (k_{cat}/K_m = 33.8 $\mu M^{-1}\cdot min^{-1}$), and also high activity for reduction of 9-*cis*-retinal to 9-*cis*-retinol (k_{cat}/K_m = 7.8 $\mu M^{-1}\cdot min^{-1}$) [27]. Hydroxysteroid substrates have not been tested.

ADH8 is found at high levels in the stomach and intestine of *R. perezi* [27], thus distinct from *Xenopus laevis* ADH4 which is expressed in stomach but not intestine [53]. ADH8 has also been identified in *Xenopus* where it is likewise expressed in the stomach and intestine (G. Duester, H. L. Ang & I. Hoffmann, unpublished data). The substrate and cofactor preferences of ADH8 as well as its expression in the digestive tract indicates that it may play a very significant role in the reduction of retinal produced by β-carotene cleavage (see Fig. 1), a reaction occurring to a large extent in the intestine [71]. Thus, ADH8 may contribute physiologically to the uptake and storage of vitamin A derived from provitamin A sources.

SHORT-CHAIN DEHYDROGENASE/REDUCTASE (SDR) FAMILY

RoDH1

A microsomal retinol dehydrogenase activity has been found in rodent liver, thus being distinct from the retinol dehydrogenase activity of ADH, which is a cytosolic enzyme. Five related forms of liver microsomal retinol dehydrogenase have been identified in rodents (RoDH1,2,3 and CRAD1,2) all sharing sequence homology with members of the SDR family and characterized by subunits of about 35 kDa with no catalytic metal ion [72–76]. RoDH1 catalyzes the oxidation of all-*trans*-retinol to all-*trans*-retinal, but cannot oxidize 9-*cis*-retinol to 9-*cis*-retinal, and prefers NADP as cofactor over NAD [72,77]. Thus, even though RoDH1 has been proposed to function in the oxidative direction, its cofactor preference with retinoids argues for a role in the reduction of all-*trans*-retinal (i.e. see below for studies supporting a role for its close homolog RoDH2 as a retinal reductase).

RoDH1 has been found to utilize all-*trans*-retinol bound to cellular retinol-binding protein type I (CRBPI), and this property has been proposed to make RoDH1 is a more 'specific' enzyme for oxidation of all-*trans*-retinol than so-called 'nonspecific dehydrogenases' including ADHs [77]. However, CRBPI sequestration of all-*trans*-retinol has also been proposed to limit ADH to metabolism of the low levels of free all-*trans*-retinol present even in the presence of CRBPI [63], thus sparing retinol from wasteful usage but still allowing a small amount to be converted to retinal for retinoic acid synthesis. Also, it should be noted that RoDH1 is itself a 'nonspecific dehydrogenase' as shown by its high activity with androgens as detailed below. In addition, the ability of CRBPI to hinder retinol oxidation is limited to only all-*trans*-retinol as CRBPI cannot bind 9-*cis*-retinol [78]. Genetic studies on knockout mice lacking CRBPI do not support a role as a facilitator of RoDH-catalyzed retinol oxidation, but instead indicate that it promotes retinol storage (esterification) and thus limits retinol oxidation as mice survive and reproduce but have depleted stores of liver retinyl esters and slightly higher levels of retinoic acid [79]. Those studies also demonstrated that CRBPI knockout mice maintained on a vitamin-A-deficient diet undergo the symptoms of hypovitaminosis A much sooner than wild-type mice by fully exhausting their retinyl ester stores.

Interestingly, RoDH1 was also isolated as an enzyme that efficiently catalyzes oxidation of the inactive androgen 5α-androstane-3α,17β-diol (3α-diol) to the active form dihydrotestosterone (V_{max}/K_m = 5.0 $nmol\cdot min^{-1}\cdot mg^{-1}\cdot \mu M^{-1}$) [80]. RoDH1 has 25-fold higher activity for 3α-diol than for all-*trans*-retinol bound to CRBPI (V_{max}/K_m = 0.2 $nmol\cdot min^{-1}\cdot mg^{-1}\cdot \mu M^{-1}$) [77] and with androgen substrates it prefers NAD over NADP as expected for catalysis of an oxidative reaction [80].

RoDH1 has been found in the mouse and rat [76], but no homolog was found in humans when investigated [81]; no homologs have yet been described in other vertebrate species. RoDH1 expression is found mostly in the adult rodent liver, but was also reported to exist at very low levels in kidney, lung, testis, and brain [28,72,80]. The lack of evolutionary conservation of RoDH1 and its ability to metabolize 3α-hydroxysteroid androgens 25-fold more efficiently than all-*trans*-retinol suggests that although this enzyme may play a species-specific role in retinoid metabolism, it may play a larger role in rodent androgen metabolism.

RoDH2

Another microsomal SDR, RoDH2, which has 82% sequence identity to RoDH1, has been found to catalyze the oxidation of all-*trans*-retinol to all-*trans*-retinal with NADP as the preferred cofactor over NAD [73]. RoDH2 is expressed mostly in liver and kidney with trace amounts in lung, brain, and testis [73]. RoDH2 homologs have been found in mouse and rat [76], but not in humans or other vertebrates. RoDH2 has been found to function efficiently in the reduction of all-*trans*-retinal to all-*trans*-retinol with NADPH as cofactor (3 $nmol\cdot min^{-1}\cdot mg^{-1}$), and this activity is stimulated by the addition of the cytochrome P450 CYP2D1 (7 $nmol\cdot min^{-1}\cdot mg^{-1}$) [82]. RoDH2 and CYP2D1 have been shown to form a stable complex [82], and during the initial purification of RoDH it was isolated as a stable complex with a 54-kDa protein [77], that being the size of CYP2D1. Thus, the available evidence points strongly to RoDH2 functioning with CYP2D1 as a retinal reductase for retinoid storage rather than in the oxidation of retinol for production of retinoic acid.

RoDH3

A third microsomal SDR, RoDH3, which has 98% sequence identity to RoDH1, has been reported in the rat [74], but homologs in mouse, humans, or other vertebrates have not been found. This enzyme is expressed only in adult liver [74]. Other properties have not been reported, but are likely to be similar to RoDH1.

RoDH4

A human microsomal SDR RoDH4 has been described that catalyzes the oxidation of all-*trans* retinol to all-*trans*-retinal, but it was suggested that RoDH4 is not the human ortholog of rodent RoDH1, 2, or 3 as NAD is the preferred cofactor over NADP and the sequence identity is only 73% [81]. As is the case for RoDH1, RoDH4 is much more efficient in the oxidation of 3α-hydroxysteroid androgens (V_{max}/K_m = 31 $nmol\cdot min^{-1}\cdot mg^{-1}\cdot \mu M^{-1}$) than in the oxidation of all-*trans* retinol (V_{max}/K_m not reported) [81]. RoDH4 expression was initially reported to exist only in adult liver,

fetal liver and fetal lung [81], but was later found also in human epidermis where it was called hRDH-E [83]. Activity studies of cells transfected with epidermal RoDH4 indicated 15-fold higher activity with the androgen 3α-diol (3.1 nmol·mg^{-1}·h^{-1}) than with all-*trans* retinol (0.2 nmol·mg^{-1}·h^{-1}) [83].

CRAD1

CRAD1 is a distinct microsomal SDR identified so far only in the mouse (80% sequence identity to RoDH1) that has been reported to catalyze the NAD-dependent oxidation of *cis*-retinols much more efficiently than *trans*-retinols, i.e. oxidation of 9-*cis*-retinol or 11-*cis*-retinol to 9-*cis*-retinal or 11-*cis*-retinal [75]. However, CRAD1 has 60-fold higher activity with the androgen 3α-diol (V_{max}/K_m = 135 nmol·min^{-1}·mg^{-1}·μM^{-1}) than with 9-*cis*-retinol or 11-*cis*-retinol which behave similarly (V_{max}/K_m = 2.3 nmol·min^{-1}·mg^{-1}·μM^{-1}) [75]. The much greater activity with androgens makes it unclear whether it also plays a major role in retinoid metabolism. CRAD1 expression is primarily in kidney, liver, and heart with trace amounts in lung, brain, and testis [75].

CRAD2

Another microsomal SDR identified so far only in the mouse is CRAD2 (82% sequence identity to RoDH1) that also has much higher activity with 3α-hydroxysteroids than with retinoids, but which shows much higher activity for 11-*cis*-retinol than for 9-*cis*-retinol or all-*trans* retinol [76]. CRAD2 has 40-fold higher activity with 3α-diol (V_{max}/K_m = 1.6 nmol·min^{-1}·mg^{-1}·μM^{-1}) than with 11-*cis*-retinol (V_{max}/K_m = 0.04 nmol·min^{-1}·mg^{-1}·μM^{-1}) [76]. Compared with the studies described above on CRAD1, these findings indicate that CRAD2 is 60-fold less efficient than CRAD1 with retinoid substrates. This combined with its more favorable kinetic properties with androgen substrates suggests CRAD2 does not play a significant role in retinoid metabolism. CRAD2 is expressed primarily in liver with trace amounts in lung, kidney, and brain [76].

RDH5

A microsomal 11-*cis*-retinol dehydrogenase present in the bovine pigment epithelium believed to function in 11-*cis*-retinal production for the visual cycle was shown to be a member of the SDR family [84,85]. This SDR was shown to be in a family quite distinct from the RoDH/CRAD family with which it shares only about 52% sequence identity [86]. The human SDR homolog sharing 91% sequence identity to the bovine form was named RDH5 [87,88]; this human SDR was independently isolated and named 9cRDH due to its ability to use 9-*cis*-retinol as a substrate [86], although it was later found to use both 11-*cis*-retinol and 9-*cis*-retinol [89]. A mouse SDR homolog with 86% sequence identity to the bovine form was found to use both 11-*cis*-retinol and 9-*cis*-retinol, but was given the name RDH4 as it was thought to possibly represent a gene distinct from RDH5 due to expression outside the eye in several adult and embryonic tissues [90]. This same mouse SDR was independently isolated and named 11-*cis*-RoDH by one group because expression in the eye as well as other sites was verified [91], and named cRDH by another group because it was found to use both 11-*cis*-retinol and 9-*cis*-retinol [89]. The sequence homologies among these human, bovine, and mouse enzymes clearly indicate that all represent one gene called RDH5 here, and direct comparisons of their activities indicate that they are all indeed the same enzyme [92].

RDH5 can produce the visual pigment 11-*cis*-retinal by NAD-dependent oxidation of 11-*cis*-retinol, and is equally effective in the oxidation of 9-*cis*-retinol to 9-*cis*-retinal (V_{max}/K_m = 3.5 nmol·min^{-1}·mg^{-1}·μM^{-1}), but has very low activity with all-*trans*-retinol (V_{max}/K_m not reported); activity with the androgen 3α-diol (V_{max}/K_m = 1.4 nmol·min^{-1}·mg^{-1}·μM^{-1}) is less than that seen with retinoids [88]. In another study, human and mouse RDH5 were both found to have the highest activity with 11-*cis* and 9-*cis*-retinol, and both had low activity with all-*trans*-retinol amounting to about 20% the activity seen with *cis*-retinols, but neither had activity with androgens [89]. One major finding from these studies is that RDH5 may be able to participate in the production of 9-*cis*-retinal for synthesis of 9-*cis*-retinoic acid as well as function in the visual cycle. This is supported by reports of RDH5 expression in several retinoid-responsive adult tissues outside the pigment epithelium [86,88,91] and expression in mouse embryos [90].

Genetic evidence supporting the role of RDH5 in 11-*cis*-retinal production has come from the identification of mutations in the human RDH5 gene that cause delayed dark adaptation and fundus albipunctatus, correlated with decreased activity of RDH5 for 11-*cis*-retinol oxidation [93].

retSDR1

Another microsomal SDR functioning in the visual cycle is retSDR1, which prefers NADPH as cofactor and catalyzes reduction of all-*trans*-retinal to all-*trans*-retinol in the retina [94]. retSDR1 lies in a distinct class of SDR as it has no 3α-hydroxysteroid activity and it shares only 26–29% sequence identity with the RoDH/CRAD family and 29% with RDH5. The reaction catalyzed by retSDR1 is essential for completion of the visual cycle in higher animals as a tremendous amount of 11-*cis*-retinal is isomerized to all-*trans*-retinal during photo bleaching of rhodopsin and then released into the retina where it must be metabolized to all-*trans*-retinol and then transported to the pigment epithelium to regenerate 11-*cis*-retinal. Mouse, human, and bovine forms of retSDR1 have been identified (human and mouse forms share 94% amino-acid sequence identity) and expression is highest in cone photoreceptor cells of the retina [94].

ALDEHYDE DEHYDROGENASE (ALDH) FAMILY

ALDH1

ALDH consists of a large family of enzymes containing some members able to use retinoids as substrates [29]. The original cytosolic class I ALDHs identified as being able to oxidize retinal to retinoic acid were called ALDH1 in the human [95], Ahd2 in the mouse [96], and RALDH or RalDHI in the rat [97,98]. The long-standing human name ALDH1 has been adopted here for all these mammalian orthologs as well as those known to exist in the chick [99] and frog [100]. ALDH1 catalyzes the NAD-dependent oxidation of both all-*trans*-retinal and 9-*cis*-retinal to all-*trans*-retinoic acid and 9-*cis*-retinoic acid with nearly equal efficiency [97]. Thus, ALDH1 can participate in production of retinoids needed to bind either RAR or RXR to regulate gene expression. The tertiary structure of ALDH1 has demonstrated that it possesses an active site that can easily accommodate all-*trans*-retinal [101].

ALDH1 is a highly conserved protein with human and mouse forms sharing 87% amino-acid sequence identity [102]; mouse

and frog forms share 76% identity [100]. ALDH1 expression is conserved in embryos and several adult epithelial tissues of both the mouse [103] and frog [104]. Expression of ALDH1 in the dorsal retina has been associated with retinoic acid synthesis in that tissue [105]. Genetic studies in *Xenopus* embryos have shown that overexpression of ALDH1 leads to premature synthesis of retinoic acid, thus providing evidence that ALDH1 can indeed function to produce retinoic acid *in vivo* [100].

ALDH6

A unique cytosolic ALDH activity present in the mouse embryonic ventral retina (V1 enzyme) is associated with retinoic acid synthesis in that tissue [105]. However, the ALDH family member responsible has not previously been reported. Human ALDH6 is distinct cytosolic ALDH sharing 70% sequence identity with ALDH1 [106] that has been shown to catalyze the oxidation of all-*trans*-retinal to all-*trans*-retinoic acid [29]. *In situ* hybridization studies show that the human ALDH6 cDNA can detect *Aldh6* mRNA in the ventral retina of mouse and chick embryos (G. Duester & M. Maden, unpublished data). This indicates that ALDH6 most likely represents the V1 enzyme, a conserved retinoic acid synthetic enzyme in the ventral retina.

RALDH2

RALDH2 (originally called V2 enzyme of mouse embryonic retina or RalDHII of rat testis) represents another cytosolic ALDH (72% sequence identity to ALDH1) able to catalyze the NAD-dependent oxidation of all-*trans*-retinal to all-*trans*-retinoic acid [107,108]. RALDH2 homologs cloned from human [109] and chick [110] have revealed amino-acid sequence identities of 98 and 94% with the mouse form, respectively, showing that RALDH2 is remarkably well conserved during evolution. The catalytic activity of RALDH2 with all-*trans*-retinal (V_{max}/K_m = 49 nmol·min^{-1}·mg^{-1}·μM^{-1}) [108] is about 15-fold higher than that of ALDH1 (V_{max}/K_m = 3.3 nmol·min^{-1}·mg^{-1}·μM^{-1}) [111]. The crystal structure of RALDH2 indicates the presence of a disordered loop in its active site that accommodates all-*trans*-retinal in a different manner than that seen for ALDH1 [112]. RALDH2 is expressed in mouse adult reproductive organs and embryos [103,113] as well as in chick embryos [110,114]. Overexpression of RALDH2 in *Xenopus* embryos leads to high levels of retinoic acid synthesis [103]. *Raldh2* knockout mice suffer from embryonic lethality at E10.5 and display an almost total lack of retinoic acid, thus providing genetic evidence that RALDH2 indeed functions to produce retinoic acid for embryonic growth and development [115].

ALDH-t

Tunicates are invertebrate chordates that have been reported to have endogenous all-*trans*-retinal and all-*trans*-retinoic acid as well as an ALDH able to oxidize retinal to retinoic acid in developing bud tissues produced during asexual reproduction [16]. This ALDH has not been purified or cloned and is referred to here as ALDH-t. Tunicates also possesses an RAR clearly related to vertebrate RARs [18]. The presence of both a retinoic acid synthetic enzyme and a retinoic acid receptor in tunicates indicates that primitive chordate animals have already evolved a retinoid signaling pathway controlling growth and development [16]. In contrast, there is no evidence that nonchordate animals such as insects or worms have evolved the ability to produce retinoic acid from vitamin A and use it to control growth and development.

CONCLUSIONS

The compilation of retinoid dehydrogenases described here indicates the existence of five ADHs, eight SDRs, and four ALDHs. However, it is unclear if all these enzymes play significant roles in retinoid metabolism and whether their roles are conserved in evolution or species-specific. This is most evident in the identification of no less than 13 ADHs and SDRs able to catalyze retinol/retinal metabolism *in vitro*. The mouse has 12 of the 17 retinoid dehydrogenases compiled here, and eight of these 12 are conserved between mouse and human (ADH1, ADH2, ADH4, RDH5, retSDR1, ALDH1, ALDH6, and RALDH2) providing confidence that these enzymes may play significant roles in all mammals rather than species-specific roles. Several enzymes have been found so far only in mouse/rat (RoDH1, RoDH2, RoDH3, CRAD1, and CRAD2), human (RoDH4), chick (ADH7), or frog (ADH8). Further evolutionary studies are needed to determine if they have conserved or species-specific functions.

Genetic studies indicate physiological roles for two ADHs (ADH1 and ADH4) as retinol dehydrogenases to produce all-*trans*-retinal or 9-*cis*-retinal needed for retinoic acid synthesis; a physiological role for one SDR (RDH5) as a retinol dehydrogenase to produce 11-*cis*-retinal for vision; and physiological roles for two ALDHs (ALDH1 and RALDH2) as retinal dehydrogenases to produce all-*trans*-retinoic acid or 9-*cis*-retinoic needed for gene regulation. Biochemical studies suggest potentially significant roles for several other enzymes in retinoid metabolism: (a) ADH2, ADH7, RoDH1, RoDH4, and CRAD1 as retinol dehydrogenases for retinoic acid synthesis; (b) ADH8 and RoDH2 (plus possibly RoDH1) as retinal reductases to convert retinal to retinol for retinoid storage; (c) retSDR1 as a retinal reductase for completion of the visual cycle; and (d) ALDH6 as a retinal dehydrogenase to produce retinoic acid. The tunicate enzyme ALDH-t may represent an early chordate retinal dehydrogenase that was the progenitor of the vertebrate ALDH1/ALDH6/RALDH2 subfamily. There are several enzymes (RoDH1, RoDH4, CRAD1, and CRAD2) for which androgens act as the predominant substrates, thus providing impetus to examine the function of these enzymes in more detail from the perspective of androgen metabolism as well as retinoid metabolism.

ACKNOWLEDGEMENTS

This work was supported by National Institutes of Health Grants AA07261 and AA09731.

REFERENCES

1. Wald, G. (1951) The chemistry of rod vision. *Science* **113**, 287–291.
2. Maden, M. (1994) Role of retinoids in embryonic development. In *Vitamin A in Health and Disease* (Blomhoff, R., ed.), pp. 289–322. Marcel Dekker, Inc., New York.
3. Hofmann, C. & Eichele, G. (1994) Retinoids in development. In *The Retinoids: Biology, Chemistry, and Medicine* (Sporn, M.B., Roberts, A.B. & Goodman, D.S., eds), 2nd edn, pp. 387–441. Raven Press Ltd., New York.
4. Gudas, L.J., Sporn, M.B. & Roberts, A.B. (1994) Cellular biology and biochemistry of the retinoids. In *The Retinoids: Biology, Chemistry, and Medicine* (Sporn, M.B., Roberts, A.B. & Goodman, D.S., eds), 2nd edn, pp. 443–520. Raven Press, Ltd., New York.
5. Kastner, P., Chambon, P. & Leid, M. (1994) Role of nuclear retinoic

acid receptors in the regulation of gene expression. In *Vitamin A in Health and Disease* (Blomhoff, R., ed.), pp. 189–238. Marcel Dekker, Inc., New York.

6. Mangelsdorf, D.J., Umesono, K. & Evans, R.M. (1994) The retinoid receptors. In *The Retinoids: Biology, Chemistry, and Medicine* (Sporn, M.B., Roberts, A.B. & Goodman, D.S., eds), 2nd edn, pp. 319–349. Raven Press, Ltd., New York.
7. Mangelsdorf, D.J. & Evans, R.M. (1995) The RXR heterodimers and orphan receptors. *Cell* **83**, 841–850.
8. Lohnes, D., Mark, M., Mendelsohn, C., Dollé, P., Dierich, A., Gorry, P., Gansmuller, A. & Chambon, P. (1994) Function of the retinoic acid receptors (RARs) during development. (I) Craniofacial and skeletal abnormalities in RAR double mutants. *Development* **120**, 2723–2748.
9. Mendelsohn, C., Lohnes, D., Décimo, D., Lufkin, T., LeMeur, M., Chambon, P. & Mark, M. (1994) Function of the retinoic acid receptors (RARs) during development. (II) Multiple abnormalities at various stages of organogenesis in RAR double mutants. *Development* **120**, 2749–2771.
10. Sucov, H.M., Dyson, E., Gumeringer, C.L., Price, J., Chien, K.R. & Evans, R.M. (1994) RXRα mutant mice establish a genetic basis for vitamin A signaling in heart morphogenesis. *Genes Dev.* **8**, 1007–1018.
11. Kastner, P., Grondona, J.M., Mark, M., Gansmuller, A., LeMeur, M., Decimo, D., Vonesch, J.-L., Dollé, P. & Chambon, P. (1994) Genetic analysis of RXRα developmental function: Convergence of RXR and RAR signaling pathways in heart and eye morphogenesis. *Cell* **78**, 987–1003.
12. Luo, J.M., Sucov, H.M., Bader, J.A., Evans, R.M. & Giguère, V. (1996) Compound mutants for retinoic acid receptor (RAR) β and RARα1 reveal developmental functions for multiple RARβ isoforms. *Mech. Dev.* **55**, 33–44.
13. Pepe, I.M., Cugnoli, C., Peluso, M., Vergani, L. & Boero, A. (1987) Structure of a protein catalyzing the formation of 11-*cis* retinal in the visual cycle of invertebrate eyes. *Cell Biophysics* **10**, 15–22.
14. Applebury, M.L. & Hargrave, P.A. (1986) Molecular biology of the visual pigments. *Vision Res.* **26**, 1881–1895.
15. Shimeld, S.M. (1996) Retinoic acid, HOX genes and the anterior-posterior axis in chordates. *Bioessays* **18**, 613–616.
16. Kawamura, K., Hara, K. & Fujiwara, S. (1993) Developmental role of endogenous retinoids in the determination of morphallactic field in budding tunicates. *Development* **117**, 835–845.
17. Laudet, V., Auwerx, J., Gustafson, J.A. & Wahli, W. (1999) A unified nomenclature system for the nuclear receptor superfamily. *Cell* **97**, 161–163.
18. Hisata, K., Fujiwara, S., Tsuchida, Y., Ohashi, M. & Kawamura, K. (1998) Expression and function of a retinoic acid receptor in budding ascidians. *Dev. Genes Evol.* **208**, 537–546.
19. Oro, A.E., McKeown, M. & Evans, R.M. (1990) Relationship between the product of the *Drosophila ultraspiracle* locus and the vertebrate retinoid X receptor. *Nature* **347**, 298–301.
20. Jörnvall, H., Danielsson, O., Hjelmqvist, L., Persson, B. & Shafqat, J. (1995) The alcohol dehydrogenase system. *Adv. Exp. Med. Biol.* **372**, 281–294.
21. Duester, G., Farrés, J., Felder, M.R., Holmes, R.S., Höög, J.-O., Parés, X., Plapp, B.V., Yin, S.-J. & Jörnvall, H. (1999) Recommended nomenclature for the vertebrate alcohol dehydrogenase gene family. *Biochem. Pharmacol.* **58**, 389–395.
22. Jörnvall, H., Persson, B., Krook, M., Atrian, S., Gonzalez-Duarte, R., Jeffery, J. & Ghosh, D. (1995) Short-chain dehydrogenases/reductases (SDR). *Biochemistry* **34**, 6003–6013.
23. Perozich, J., Nicholas, H., Wang, B.C., Lindahl, R. & Hempel, J. (1999) Relationships within the aldehyde dehydrogenase extended family. *Protein Sci.* **8**, 137–146.
24. Persson, B., Zigler, J.S. Jr, & Jörnvall, H. (1994) A super-family of medium-chain dehydrogenases/reductases (MDR) – Sub-lines including zeta-crystallin, alcohol and polyol dehydrogenases, quinone oxidoreductases, enoyl reductases, VAT-1 and other proteins. *Eur. J. Biochem.* **226**, 15–22.
25. Baker, M.E. (1996) Unusual evolution of 11β- and 17β-hydroxysteroid and retinol dehydrogenases. *Bioessays* **18**, 63–70.
26. Lindahl, R. (1992) Aldehyde dehydrogenases and their role in carcinogenesis. *Crit. Rev. Biochem. Mol. Biol.* **27**, 283–335.
27. Peralba, J.M., Cederlund, E., Crosas, B., Moreno, A., Julià, P., Martínez, S.E., Persson, B., Farrés, J., Parés, X. & Jörnvall, H. (1999) Structural and enzymatic properties of a gastric NADP(H)-dependent and retinal-active alcohol dehydrogenase. *J. Biol. Chem.* **274**, 26021–26026.
28. Napoli, J.L. (1999) Interactions of retinoid binding proteins and enzymes in retinoid metabolism. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **1440**, 139–162.
29. Yoshida, A., Rzhetsky, A., Hsu, L.C. & Chang, C. (1998) Human aldehyde dehydrogenase gene family. *Eur. J. Biochem.* **251**, 549–557.
30. Vasiliou, V., Bairoch, A., Tipton, K.F. & Nebert, D.W. (1999) Eukaryotic aldehyde dehydrogenase (ALDH) genes: human polymorphisms, and recommended nomenclature based upon divergent evolution and chromosomal mapping. *Pharmacogenetics* **9**, 421–434.
31. Duester, G. (1996) Involvement of alcohol dehydrogenase, short-chain dehydrogenase/reductase, aldehyde dehydrogenase, and cytochrome P450 in the control of retinoid signaling by activation of retinoic acid synthesis. *Biochemistry* **35**, 12221–12227.
32. Bliss, A.F. (1951) The equilibrium between vitamin A alcohol and aldehyde in the presence of alcohol dehydrogenase. *Arch. Biochem.* **31**, 197–204.
33. Zachman, R.D. & Olson, J.A. (1961) A comparison of retinene reductase and alcohol dehydrogenase of rat liver. *J. Biol. Chem.* **236**, 2309–2313.
34. Boleda, M.D., Saubi, N., Farrés, J. & Parés, X. (1993) Physiological substrates for rat alcohol dehydrogenase classes: aldehydes of lipid peroxidation, omega-hydroxyfatty acids, and retinoids. *Arch. Biochem. Biophys.* **307**, 85–90.
35. Yang, Z.-N., Davis, G.J., Hurley, T.D., Stone, C.L., Li, T.-K. & Bosron, W.F. (1994) Catalytic efficiency of human alcohol dehydrogenases for retinol oxidation and retinal reduction. *Alcohol. Clin. Exp. Res.* **18**, 587–591.
36. Han, C.L., Liao, C.S., Wu, C.W., Hwong, C.L., Lee, A.R. & Yin, S.J. (1998) Contribution to first-pass metabolism of ethanol and inhibition by ethanol for retinol oxidation in human alcohol dehydrogenase family – implications for etiology of fetal alcohol syndrome and alcohol-related diseases. *Eur. J. Biochem.* **254**, 25–31.
37. Veech, R.L., Eggleston, L.V. & Krebs, H.A. (1969) The redox state of free nicotinamide-adenine dinucleotide phosphate in the cytoplasm of rat liver. *Biochem. J.* **115**, 609–619.
38. Popescu, G. & Napoli, J.L. (2000) Analysis of rat cytosolic 9-*cis*-retinol dehydrogenase activity and enzymatic characterization of rat ADHII. *Biochim. Biophys. Acta* **1476**, 43–52.
39. Eklund, H., Nordstrom, B., Zeppezauer, E., Soderlund, G., Ohlsson, I., Boiwe, T., Soderberg, B.-O., Tapia, O. & Branden, C.-I. (1976) Three-dimensional structure of horse liver alcohol dehydrogenase at 2.4 Å resolution. *J. Mol. Biol.* **102**, 27–59.
40. Hurley, T.D., Bosron, W.F., Stone, C.L. & Amzel, L.M. (1994) Structures of three human β alcohol dehydrogenase variants. Correlations with their functional differences. *J. Mol. Biol.* **239**, 415–429.
41. Ramaswamy, S., El Ahmad, M., Danielsson, O., Jörnvall, H. & Eklund, H. (1996) Crystal structure of cod liver class I alcohol dehydrogenase: Substrate pocket and structurally variable segments. *Protein Sci.* **5**, 663–671.
42. Moreno, A., Farrés, J., Parés, X., Jörnvall, H. & Persson, B. (1996) Molecular modelling of human gastric alcohol dehydrogenase (class IV) and substrate docking: Differences towards the classical liver enzyme (class I). *FEBS Lett.* **395**, 99–102.
43. Foglio, M.H. & Duester, G. (1999) Molecular docking studies on interaction of diverse retinol structures with human alcohol dehydrogenases predict a broad role in retinoid ligand synthesis. *Biochim. Biophys. Acta* **1432**, 239–250.
44. Okuda, A. & Okuda, K. (1983) Physiological function and kinetic

mechanism of human liver alcohol dehydrogenase as 5β-cholestane-3a,7a,12a,26-tetrol dehydrogenase. *J. Biol. Chem.* **258**, 2899–2905.

45. McEvily, A.J., Holmquist, B., Auld, D.S. & Vallee, B.L. (1988) 3β-hydroxy-5β-steroid dehydrogenase activity of human liver alcohol dehydrogenase is specific to gamma-subunits. *Biochemistry* **27**, 4284–4288.
46. Vallee, B.L. & Bazzone, T.J. (1983) Isozymes of human liver alcohol dehydrogenase. *Curr. Top. Biol. Med. Res.* **8**, 219–244.
47. Algar, E.M., Seeley, T.-L. & Holmes, R.S. (1983) Purification and molecular properties of mouse alcohol dehydrogenase isozymes. *Eur. J. Biochem.* **137**, 139–147.
48. Cederlund, E., Peralba, J.M., Parés, X. & Jörnvall, H. (1991) Amphibian alcohol dehydrogenase, the major frog liver enzyme. Relationships to other forms and assessment of an early gene duplication separating vertebrate class I and class III alcohol dehydrogenases. *Biochemistry* **30**, 2811–2816.
49. Wrange, O., Okret, S., Radojcic, M., Carlstedt-Duke, J. & Gustafsson, J.-Å. (1984) Characterization of the purified activated glucocorticoid receptor from rat liver cytosol. *J. Biol. Chem.* **259**, 4534–4541.
50. Bilanchone, V., Duester, G., Edwards, Y. & Smith, M. (1986) Multiple mRNAs for human alcohol dehydrogenase (ADH): developmental and tissue-specific differences. *Nucleic Acids Res.* **14**, 3911–3926.
51. Ang, H.L., Deltour, L., Hayamizu, T.F., Zgombic-Knight, M. & Duester, G. (1996) Retinoic acid synthesis in mouse embryos during gastrulation and craniofacial development linked to class IV alcohol dehydrogenase gene expression. *J. Biol. Chem.* **271**, 9526–9534.
52. Ang, H.L., Deltour, L., Zgombic-Knight, M., Wagner, M.A. & Duester, G. (1996) Expression patterns of class I and class IV alcohol dehydrogenase genes in developing epithelia suggest a role for alcohol dehydrogenase in local retinoic acid synthesis. *Alcohol. Clin. Exp. Res.* **20**, 1050–1064.
53. Hoffmann, I., Ang, H.L. & Duester, G. (1998) Alcohol dehydrogenases in *Xenopus* development: conserved expression of ADH1 and ADH4 in epithelial retinoid target tissues. *Dev. Dyn.* **213**, 261–270.
54. Vonesch, J.-L., Nakshatri, H., Philippe, M., Chambon, P. & Dollé, P. (1994) Stage and tissue-specific expression of the alcohol dehydrogenase 1 (*Adh-1*) gene during mouse development. *Dev. Dyn.* **199**, 199–213.
55. Haselbeck, R.J. & Duester, G. (1997) Regional restriction of alcohol/retinol dehydrogenases along the mouse gastrointestinal epithelium. *Alcohol. Clin. Exp. Res.* **21**, 1484–1490.
56. Deltour, L., Haselbeck, R.J., Ang, H.L. & Duester, G. (1997) Localization of class I and class IV alcohol dehydrogenases in mouse testis and epididymis: Potential retinol dehydrogenases for endogenous retinoic acid synthesis. *Biol. Reprod.* **56**, 102–109.
57. Haselbeck, R.J., Ang, H.L., Deltour, L. & Duester, G. (1997) Retinoic acid and alcohol/retinol dehydrogenase in the mouse adrenal gland: a potential endocrine source of retinoic acid during development. *Endocrinology* **138**, 3035–3041.
58. Collins, M.D., Eckhoff, C., Chahoud, I., Bochert, G. & Nau, H. (1992) 4-Methylpyrazole partially ameliorated the teratogenicity of retinol and reduced the metabolic formation of all-*trans*-retinoic acid in the mouse. *Arch. Toxicol.* **66**, 652–659.
59. Deltour, L., Foglio, M.H. & Duester, G. (1999) Metabolic deficiencies in alcohol dehydrogenase *Adh1*, *Adh3*, and *Adh4* null mutant mice: Overlapping roles of *Adh1* and *Adh4* in ethanol clearance and metabolism of retinol to retinoic acid. *J. Biol. Chem.* **274**, 16796–16801.
60. Svensson, S., Strömberg, P. & Höög, J.O. (1999) A novel subtype of class II alcohol dehydrogenase in rodents – unique Pro^{47} and Ser^{182} modulates hydride transfer in the mouse enzyme. *J. Biol. Chem.* **274**, 29712–29719.
61. Cheung, C., Smith, C.K., Hoog, J.O. & Hotchkiss, S.A.M. (1999) Expression and localization of human alcohol and aldehyde dehydrogenase enzymes in skin. *Biochem. Biophys. Res. Commun.* **261**, 100–107.
62. Connor, M.J. & Smit, M.H. (1987) Terminal-group oxidation of retinol by mouse epidermis: inhibition *in vitro* and *in vivo*. *Biochem. J.* **244**, 489–492.
63. Kedishvili, N.Y., Gough, W.H., Davis, W.I., Parsons, S., Li, T.K. & Bosron, W.F. (1998) Effect of cellular retinol-binding protein on retinol oxidation by human class IV retinol alcohol dehydrogenase and inhibition by ethanol. *Biochem. Biophys. Res. Commun.* **249**, 191–196.
64. Allali-Hassani, A., Peralba, J.M., Martras, S., Farrés, J. & Parés, X. (1998) Retinoids, omega-hydroxyfatty acids and cytotoxic aldehydes as physiological substrates, and H_2-receptor antagonists as pharmacological inhibitors, of human class IV alcohol dehydrogenase. *FEBS Lett.* **426**, 362–366.
65. Xie, P.G., Parsons, S.H., Speckhard, D.C., Bosron, W.F. & Hurley, T.D. (1997) X-ray structure of human class IV σσ alcohol dehydrogenase – structural basis for substrate specificity. *J. Biol. Chem.* **272**, 18558–18563.
66. Kedishvili, N.Y., Bosron, W.F., Stone, C.L., Hurley, T.D., Peggs, C.F., Thomasson, H.R., Popov, K.M., Carr, L.G., Edenberg, H.J. & Li, T.-K. (1995) Expression and kinetic characterization of recombinant human stomach alcohol dehydrogenase. Active-site amino acid sequence explains substrate specificity compared with liver isozymes. *J. Biol. Chem.* **270**, 3625–3630.
67. Haselbeck, R.J., Ang, H.L. & Duester, G. (1997) Class IV alcohol/retinol dehydrogenase localization in epidermal basal layer: potential site of retinoic acid synthesis during skin development. *Dev. Dyn.* **208**, 447–453.
68. Julià, P., Farrés, J. & Parés, X. (1986) Ocular alcohol dehydrogenase in the rat: regional distribution and kinetics of the ADH-1 isoenzyme with retinol and retinal. *Exp. Eye Res.* **42**, 305–314.
69. Deltour, L., Foglio, M.H. & Duester, G. (1999) Impaired retinol utilization in *Adh4* alcohol dehydrogenase mutant mice. *Dev. Genet.* **25**, 1–10.
70. Kedishvili, N.Y., Gough, W.H., Chernoff, E.A.G., Hurley, T.D., Stone, C.L., Bowman, K.D., Popov, K.M., Bosron, W.F. & Li, T.K. (1997) cDNA sequence and catalytic properties of a chick embryo alcohol dehydrogenase that oxidizes retinol and 3β,5α-hydroxysteroids. *J. Biol. Chem.* **272**, 7494–7500.
71. Olson, J.A. (1989) The provitamin A function of carotenoids: the conversion of β-carotene into vitamin A. *J. Nutr.* **119**, 105–108.
72. Chai, X., Boerman, M.H.E.M., Zhai, Y. & Napoli, J.L. (1995) Cloning of a cDNA for liver microsomal retinol dehydrogenase. A tissue-specific, short-chain alcohol dehydrogenase. *J. Biol. Chem.* **270**, 3900–3904.
73. Chai, X., Zhai, Y., Popescu, G. & Napoli, J.L. (1995) Cloning of a cDNA for a second retinol dehydrogenase type II – expression of its mRNA relative to type I. *J. Biol. Chem.* **270**, 28408–28412.
74. Chai, X., Zhai, Y. & Napoli, J.L. (1996) Cloning of a rat cDNA encoding retinol dehydrogenase isozyme type III. *Gene* **169**, 219–222.
75. Chai, X.Y., Zhai, Y. & Napoli, J.L. (1997) cDNA cloning and characterization of a *cis*-retinol/3α-hydroxysterol short-chain dehydrogenase. *J. Biol. Chem.* **272**, 33125–33131.
76. Su, J., Chai, X.Y., Kahn, B. & Napoli, J.L. (1998) cDNA cloning, tissue distribution, and substrate characteristics of a *cis*-retinol/3α-hydroxysterol short-chain dehydrogenase isozyme. *J. Biol. Chem.* **273**, 17910–17916.
77. Boerman, M.H.E.M. & 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.
78. MacDonald, P.N. & Ong, D.E. (1987) Binding specificities of cellular retinol-binding protein and cellular retinol-binding protein, type II. *J. Biol. Chem.* **262**, 10550–10556.
79. Ghyselinck, N.B., Båvik, C., Sapin, V., Mark, M., Bonnier, D., Hindelang, C., Dierich, A., Nilsson, C.B., Håkansson, H., Sauvant, P., Azaïs-Braesco, V., Frasson, M., Picaud, S. & Chambon, P. (1999) Cellular retinol-binding protein I is essential for vitamin A homeostasis. *EMBO J.* **18**, 4903–4914.
80. Biswas, M.G. & Russell, D.W. (1997) Expression cloning and characterization of oxidative 17β- and 3α-hydroxysteroid

dehydrogenases from rat and human prostate. *J. Biol. Chem.* **272**, 15959–15966.
81. Gough, W.H., VanOoteghem, S., Sint, T. & Kedishvili, N.Y. (1998) cDNA cloning and characterization of a new human microsomal NAD^+-dependent dehydrogenase that oxidizes all-*trans*-retinol and 3α-hydroxysteroids. *J. Biol. Chem.* **273**, 19778–19785.
82. Imaoka, S., Wan, J., Chow, T., Hiroi, T., Eyanagi, R., Shigematsu, H. & Funae, Y. (1998) Cloning and characterization of the CYP2D1-binding protein, retinol dehydrogenase. *Arch. Biochem. Biophys.* **353**, 331–336.
83. Jurukovski, V., Markova, N.G., Karaman-Jurukovska, N., Randolph, R.K., Su, J., Napoli, J.L. & Simon, M. (1999) Cloning and characterization of retinol dehydrogenase transcripts expressed in human epidermal keratinocytes. *Mol. Genet. Metabolism* **67**, 62–73.
84. Simon, A., Hellman, U., Wernstedt, C. & Eriksson, U. (1995) The retinal pigment epithelial-specific 11-*cis* retinol dehydrogenase belongs to the family of short chain alcohol dehydrogenases. *J. Biol. Chem.* **270**, 1107–1112.
85. Driessen, C.A.G.G., Janssen, B.P.M., Winkens, H.J., Van Vugt, A.H.M., Leeuw, T.L.M. & Janssen, J.J.M. (1995) Cloning and expression of a cDNA encoding bovine retinal pigment epithelial 11-*cis* retinol dehydrogenase. *Invest. Ophthalmol. Vis. Sci.* **36**, 1988–1996.
86. Mertz, J.R., Shang, E.Y., Piantedosi, R., Wei, S.H., Wolgemuth, D.J. & Blaner, W.S. (1997) Identification and characterization of a stereospecific human enzyme that catalyzes 9-*cis*-retinol oxidation – a possible role in 9-*cis*-retinoic acid formulation. *J. Biol. Chem.* **272**, 11744–11749.
87. Simon, A., Lagercrantz, J., Bajalica-Lagercrantz, S. & Eriksson, U. (1996) Primary structure of human 11-*cis* retinol dehydrogenase and organization and chromosomal localization of the corresponding gene. *Genomics* **36**, 424–430.
88. Wang, J., Chai, X.Y., Eriksson, U. & Napoli, J.L. (1999) Activity of human 11-*cis*-retinol dehydrogenase (Rdh5) with steroids and retinoids and expression of its mRNA in extra-ocular human tissue. *Biochem. J.* **338**, 23–27.
89. Gamble, M.V., Shang, E.Y., Zott, R.P., Mertz, J.R., Wolgemuth, D.J. & Blaner, W.S. (1999) Biochemical properties, tissue expression, and gene structure of a short chain dehydrogenase/reductase able to catalyze *cis*-retinol oxidation. *J. Lipid Res.* **40**, 2279–2292.
90. Romert, A., Tuvendal, P., Simon, A., Dencker, L. & Eriksson, U. (1998) The identification of a 9-*cis* retinol dehydrogenase in the mouse embryo reveals a pathway for synthesis of 9-*cis* retinoic acid. *Proc. Natl Acad. Sci. USA* **95**, 4404–4409.
91. Driessen, C.A.G.G., Winkens, H.J., Kuhlmann, E.D., Janssen, A.P.M., Van Vugt, A.H.M., Deutmann, A.F. & Janssen, J.J.M. (1998) The visual cycle retinol dehydrogenase: possible involvement in the 9-*cis* retinoic acid biosynthetic pathway. *FEBS Lett.* **428**, 135–140.
92. Gamble, M.V., Mata, N.L., Tsin, A.T., Mertz, J.R. & Blaner, W.S. (2000) Substrate specificities and 13-*cis*-retinoic acid inhibition of human, mouse and bovine *cis*-retinol dehydrogenases. *Biochim. Biophys. Acta* **1476**, 3–8.
93. Yamamoto, H., Simon, A., Eriksson, U., Harris, E., Berson, E.L. & Dryja, T.P. (1999) Mutations in the gene encoding 11-*cis* retinol dehydrogenase cause delayed dark adaptation and fundus albipunctatus. *Nat. Genet.* **22**, 188–191.
94. Haeseleer, F., Huang, J., Lebioda, L., Saari, J.C. & Palczewski, K. (1998) Molecular characterization of a novel short-chain dehydrogenase/reductase that reduces all-*trans*-retinal. *J. Biol. Chem.* **273**, 21790–21799.
95. Dockham, P.A., Lee, M.-O. & Sladek, N.E. (1992) Identification of human liver aldehyde dehydrogenases that catalyze the oxidation of aldophosphamide and retinaldehyde. *Biochem. Pharmacol.* **43**, 2453–2469.
96. Lee, M.-O., Manthey, C.L. & Sladek, N.E. (1991) Identification of mouse liver aldehyde dehydrogenases that catalyze the oxidation of retinaldehyde to retinoic acid. *Biochem. Pharmacol.* **42**, 1279–1285.
97. Labrecque, J., Dumas, F., Lacroix, A. & Bhat, P.V. (1995) A novel isoenzyme of aldehyde dehydrogenase specifically involved in the biosynthesis of 9-*cis* and all-*trans* retinoic acid. *Biochem. J.* **305**, 681–684.
98. Penzes, P., Wang, X.S., Sperkova, Z. & Napoli, J.L. (1997) Cloning of a rat cDNA encoding retinal dehydrogenase isozyme type I and its expression in *E. coli*. *Gene* **191**, 167–172.
99. Godbout, R. (1992) High levels of aldehyde dehydrogenase transcripts in the undifferentiated chick retina. *Exp. Eye Res.* **54**, 297–305.
100. Ang, H.L. & Duester, G. (1999) Stimulation of premature retinoic acid synthesis in *Xenopus* embryos following premature expression of aldehyde dehydrogenase ALDH1. *Eur. J. Biochem.* **260**, 227–234.
101. Moore, S.A., Baker, H.M., Blythe, T.J., Kitson, K.E., Kitson, T.M. & Baker, E.N. (1998) Sheep liver cytosolic aldehyde dehydrogenase: the structure reveals the basis for the retinal specificity of class 1 aldehyde dehydrogenases. *Structure* **6**, 1541–1551.
102. Hsu, L.C., Chang, W.C., Hoffmann, I. & Duester, G. (1999) Molecular analysis of two closely related mouse aldehyde dehydrogenase genes: identification of a role for *Aldh1*, but not *Aldh-pb*, in the biosynthesis of retinoic acid. *Biochem. J.* **339**, 387–395.
103. Haselbeck, R.J., Hoffmann, I. & Duester, G. (1999) Distinct functions for *Aldh1* and *Raldh2* in the control of ligand production for embryonic retinoid signaling pathways. *Dev. Genet.* **25**, 353–364.
104. Ang, H.L. & Duester, G. (1999) Retinoic acid biosynthetic enzyme ALDH1 localizes in a subset of retinoid-dependent tissues during *Xenopus* development. *Dev. Dyn.* **215**, 264–272.
105. McCaffery, P., Wagner, E., O'Neil, J., Petkovich, M. & Dräger, U.C. (1999) Dorsal and ventral retinal territories defined by retinoic acid synthesis, break-down and nuclear receptor expression. *Mech. Dev.* **82**, 119–130.
106. Hsu, L.C., Chang, W.-C., Hiraoka, L. & Hsieh, C.-L. (1994) Molecular cloning, genomic organization, and chromosomal localization of an additional human aldehyde dehydrogenase gene, *ALDH6*. *Genomics* **24**, 333–341.
107. Zhao, D., McCaffery, P., Ivins, K.J., Neve, R.L., Hogan, P., Chin, W.W. & Dräger, U.C. (1996) Molecular identification of a major retinoic-acid-synthesizing enzyme, a retinaldehyde-specific dehydrogenase. *Eur. J. Biochem.* **240**, 15–22.
108. Wang, X.S., Penzes, P. & Napoli, J.L. (1996) Cloning of a cDNA encoding an aldehyde dehydrogenase and its expression in *Escherichia coli* – recognition of retinal as substrate. *J. Biol. Chem.* **271**, 16288–16293.
109. Ono, Y., Fukuhara, N. & Yoshie, O. (1998) TAL1 and LIM-only proteins synergistically induce retinaldehyde dehydrogenase 2 expression in T-cell acute lymphoblastic leukemia by acting as cofactors for GATA3. *Mol. Cell. Biol.* **18**, 6939–6950.
110. Swindell, E.C., Thaller, C., Sockanathan, S., Petkovich, M., Jessell, T.M. & Eichele, G. (1999) Complementary domains of retinoic acid production and degradation in the early chick embryo. *Dev. Biol.* **216**, 282–296.
111. Penzes, P., Wang, X.S. & Napoli, J.L. (1997) Enzymatic characteristics of retinal dehydrogenase type I expressed in *Escherichia coli*. *Biochim. Biophys. Acta* **1342**, 175–181.
112. Lamb, A.L. & Newcomer, M.E. (1999) The structure of retinal dehydrogenase type II at 2.7 Å resolution: Implications for retinal specificity. *Biochemistry* **38**, 6003–6011.
113. Niederreither, K., McCaffery, P., Dräger, U.C., Chambon, P. & Dollé, P. (1997) Restricted expression and retinoic acid-induced downregulation of the retinaldehyde dehydrogenase type 2 (RALDH-2) gene during mouse development. *Mech. Dev.* **62**, 67–78.
114. Berggren, K., McCaffery, P., Dräger, U. & Forehand, C.J. (1999) Differential distribution of retinoic acid synthesis in the chicken embryo as determined by immunolocalization of the retinoic acid synthetic enzyme, RALDH-2. *Dev. Biol.* **210**, 288–304.
115. Niederreither, K., Subbarayan, V., Dollé, P. & Chambon, P. (1999) Embryonic retinoic acid synthesis is essential for early mouse post-implantation development. *Nat. Genet.* **21**, 444–448.