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Physiological insights into all-*trans*-retinoic acid biosynthesis

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Abstract

All-*trans*-retinoic acid (atRA) provides essential support to diverse biological systems and physiological processes. Epithelial differentiation and its relationship to cancer and embryogenesis have typified intense areas of interest into atRA function. Recently, however, interest in atRA action in the nervous system, the immune system, energy balance and obesity has increased considerably, especially concerning postnatal function. atRA action depends on atRA biosynthesis: defects in retinoid-dependent processes increasingly relate to defects in atRA biogenesis. Considerable evidence indicates that physiological atRA biosynthesis occurs via a regulated process, consisting of a complex interaction of retinoid binding-proteins and retinoid recognizing enzymes. An accrual of biochemical, physiological and genetic data have identified

[1]The abbreviations used are:

ADH	medium-chain alcohol dehydrogenase(s)
ALDH	aldehyde dehydrogenase(s)
ARAT	acyl-CoA:retinol acyltransferase
atRA	all-*trans*-retinoic acid
CRABP	cellular retinoic acid binding-protein(s)
CRBP	cellular retinol binding-protein(s)
FABP	fatty acid binding protein
LRAT	lecithin:retinol acyltransferase
RAR	retinoic acid receptor(s)
RBP	serum retinol binding-protein
RDH	retinol dehydrogenase
RE	retinyl ester(s)
REH	retinyl ester hydrolase
RRD	retinal reductase
SDR	short-chain dehydrogenase/reductase
STRA	(gene) stimulated by RA
WT	wild-type

specific functional outcomes for the retinol dehydrogenases, RDH1, RDH10, and DHRS9, as physiological catalysts of the first step in atRA biosynthesis, and for the retinal dehydrogenases RALDH1, RALDH2, and RALDH3, as catalysts of the second and irreversible step. Each of these enzymes associates with explicit biological processes mediated by atRA. Redundancy occurs, but seems limited. Cumulative data supports a model of interactions among these enzymes with retinoid binding-proteins, with feedback regulation and/or control by atRA via modulating gene expression of multiple participants. The ratio apo-CRBP1/holo-CRBP1 participates by influencing retinol flux into and out of storage as retinyl esters, thereby modulating substrate to support atRA biosynthesis. atRA biosynthesis requires presence of both an RDH and an RALDH: conversely, absence of one isozyme of either step does not indicate lack of atRA biosynthesis at the site.

Keywords

retinol; retinoic acid; aldehyde dehydrogenase; cellular retinoic acid binding-protein; cellular retinol binding-protein; lecithin:retinol acyltransferase; retinol dehydrogenase; retinyl ester; retinal reductase; short-chain dehydrogenase/reductase

1. Introduction

Multiple functions of all-*trans*-retinoic acid (atRA) in diverse physiological systems have engendered interest beyond the traditional retinoid attention areas of cancer and development. Interest in atRA action has increased considerably concerning the nervous system, the immune system, energy balance and obesity, to name a few. Curiosity also has increased in the physiological biogeneration of atRA, because atRA biosynthesis contributes to regulating atRA action, and defects in atRA biosynthesis likely contribute to defects in retinoid action. Considerable evidence indicates that physiological atRA biosynthesis occurs via a regulated process, consisting of a complex interaction of retinoid binding-proteins and retinoid recognizing enzymes, quite distinct from the unregulated production of atRA pursuant to an excess of retinol, which is rarely observed in nature. Although vitamin A intake appears adequate in most (but not all) populations of developed countries, this likely represents an evolutionarily aberrant occurrence. As indicated by widespread deficiency in developing or underdeveloped countries, limited vitamin A intake seems the norm, resulting from nutritionally poor diets [1]. Thus, atRA generation must be considered in the context of the physiological forms of its precursors, retinol bound with cellular retinol binding-proteins, and the struggle to obtain and conserve an essential nutrient. The retinoid binding-proteins likely have multiple functions, including aiding in cellular uptake of retinol and protecting retinol from metabolism by xenobiotic clearing enzymes. In fact, at least two examples of retinoid knockouts are known that produced no phenotype when mice were fed a chow diet (contains copious vitamin A), but showed a distinctive retinoid-related phenotype when dietary vitamin A was lowered: *CRBP2* and *RDH1* [2, 3]. This indicates that these proteins evolved (in part) to help animals cope with restricted dietary vitamin A by maximizing its uptake and use, and shows that diets copious in vitamin A or pharmacological dosing with retinol eliminates a primary purpose for expressing these proteins. This review will focus on the biochemistry of atRA biosynthesis under physiological conditions, the evidence for involvement of retinoid binding-proteins in retinol metabolism, and the evidence for participation of specific enzymes. The data will be presented in context of the entire atRA generating “metabolon” and will consider regulatory nodes in an effort to present a model that integrates the functions of retinoid binding-proteins and enzymes that catalyze retinoid metabolism with their biochemistry and physiological functions.

2. An integrative model of physiological atRA homeostasis

A problem with studying retinoid metabolism is the promiscuity and lability of retinoids in vitro, as well as the promiscuity of xenobiotic clearing enzymes, and even many enzymes assumed to be specific. Simply because a known enzyme has activity with retinoids in vitro does not indicate whether it has access in vivo. The overview in Figure 1 formulates a model developed largely via “de novo” approaches to delineate atRA homeostasis, i.e. by searching tissues for retinoid metabolizing enzymes with physiological amounts and forms of substrate.

3. Retinol homeostasis

Liver stores the largest amount of vitamin A, delivered on chylomicron remnants, as retinyl esters (RE), and releases retinol bound with the serum retinol binding-protein, RBP (encoded by *Rbp4*) into circulation [4-8]. RBP interacts with the extra-hepatic plasma membrane receptor STRA6, which mediates transfer of retinol into cells [9]. STRA6 enhances LRAT activity by delivering substrate [10]. The converse also is true: net transfer mediated by STRA6 occurs only if cells express either CRBP1 (encoded by *Rbp1*), and/or LRAT[2]. This insight complements observation of coupled retinol uptake and esterification, and of holo-CRBP1 (retinol bound with CRBP1) serving as a chaperone for retinol esterification [11-13]. This perspective also reflects holo-CRBP1 occurring as the most abundant form of intracellular non-esterified retinol, despite capacity for membranes to sequester far more retinol than occurs in cells during normal vitamin A nutriture [14]. The total CRBP1 concentration remains steady with varying vitamin A status, observed when feeding rats diets devoid of vitamin A vs. feeding diets containing ~2.5-fold more than the American Institute of Nutrition (AIN) recommended concentration for rodents (equivalent to 1.2 μg retinol = 4 IU/g diet) [15, 16]. The retinol concentration also remains within limited bounds with changes in dietary vitamin A, from a diet containing the 4 IU vitamin A/g diet recommended by the AIN, up to a single dosing with a copious amount of retinol (~20 mg/kg, >66,000 IU). In contrast, the amount of RE increases substantially with increasing dietary vitamin A [17]. The mouse recapitulates this phenomenon. As dietary vitamin A increases from marginal (0.6 IU vitamin A/g diet) to the AIN recommended amount, the liver retinol concentration increases 2-fold, but RE increase 6-fold [3]. Accumulation of RE in liver and other tissues allows for retinol storage in an innocuous form in lipid droplets, and for repetitive use of CRBP1, maximizing retinol uptake and storage, while minimizing need for expressing larger concentrations of CRBP1 (Figure 1).

Kinetic analysis reveals that LRAT (in its native association with microsomal membranes) accesses retinol bound to CRBP1 [13, 18]. RE formation in vitro occurs in the presence of holo-CRBP1 at a rate faster than allowed by the low concentration of “free” retinol in solution that would equilibrate with holo-CRBP1, assuming any substantial retinol actually can occur in a aqueous solution. The latter is highly unlikely—retinol solubility in aqueous medium does not exceed ~60 nM in the absence of CRBP1 [19]. In the presence of μM holo-CRBP1 with CRBP1 in excess, the law of mass action predicts nM retinol concentrations. The rate of RE formation in the presence of holo-CRBP1 very much exceeds the rate that would be supported if unbound retinol were the only substrate, and the relationship between holo-CRBP1 and RE formation displays Michaelis-Menten kinetics.

Although LRAT catalyzes RE biosynthesis in most tissues, an alternative activity, ARAT, occurs in some tissues, notably in the mammary gland and skin [20-23]. In skin, the enzyme

[2]Hui Sun, personal communication.

DGAT1 (diacylglyceracyltransferase) accounts for ARAT activity [24]. The physiological advantage of requiring a second retinol esterifying enzyme has not been determined.

Stored RE require mobilization into retinol to support vitamin A function. Several classes of membrane-associated lipases convert RE into retinol, and each may be specific for a specific phase of vitamin A distribution, such as hydrolysis of chylomicron or chylomicron remnant-containing RE or endosomal packaged RE [25, 26]. A bile-salt independent neutral REH seems physiologically relevant for generating retinol from intracellular stores [27-31]. This deduction is supported by the microsomal localization of the hydrolase, i.e. intracellular, and the ability of apo-CRBP1 to stimulate hydrolysis of endogenous microsomal RE via the bile salt independent REH [32]. Alternative proteins that can bind retinol, such as albumin and β-lactoglobulin, and another member of the FABP gene family that binds atRA but not retinol, CRABP1, do not have the same effect. Thus, RE hydrolysis reacts to apo-CRBP1 specifically. Stimulation of RE hydrolysis by apo-CRBP1 conforms to Michaelis-Menten kinetics: it is saturable and 2.6 μM apo-CRBP1, a concentration in the range of the physiological concentration in liver, produces half-maximal stimulation (Figure 2).

In contrast to stimulation of RE hydrolysis, apo-CRBP1 inhibits retinol esterification by LRAT with a inhibitory constant of ~0.2 μM [33]. This suggests that the ratio holo-CRBP1/apo-CRBP1 serves as a signal of retinol status, which directs flux of retinol into and out of storage as RE, while maintaining a steady-state concentration of holo-CRBP1 to support atRA biosynthesis. As mentioned above, RE represent the major disposition of retinol upon entering the liver. As the cell accumulates retinol, the RE concentration increases far more than the retinol concentration. Ability of apo-CRBP1 to inhibit LRAT activity would prevent all retinol from converting (or re-converting) into RE. RE production would continue as long as retinol continued to enter the liver. But as retinol presentation diminishes, LRAT action would deplete holo-CRBP1 of retinol, generating apo-CRBP1 and limiting the rate of esterification, while prompting mobilization of RE. This seems an elegant mechanism to insure that not all retinol ends up as RE, to provide substrate for atRA biosynthesis, and to prevent futile cycling of retinol back and forth into and out of RE. Presumably, the ratio apo-CRBP1/holo-CRBP1 functions extrahepatically, as it seems to function in liver. Retinol concentrations in mouse tissues other than liver range from 0.2 to 0.6 μM, whereas RE concentrations range from 0.2 to 2 μM, in mice fed the recommended amounts of vitamin A [34]. As a rule of thumb, RE concentrations exceed those of retinol in these tissues, but the differences are not as large as in liver.

4. CRBP1 as a chaperone

Although affinity of CRBP1 for retinol had been estimated as ~16 nM, this value has been re-determined as 3 nM, by availability of a more sensitive fluorometer [35-37]. Both values likely represent an upper limit rather than a true value, because fluorometer sensitivity has limited the generation of apparent k_d values for retinoid binding-proteins. Estimates of the true value approach 0.1 nM, based on competition experiments, rather than fluorescence quenching [38]. Sequestration of retinol *inside* a high-affinity binding-protein draws retinol into the cell and protects it from unfettered metabolism, but clearly allows RE formation [36, 39]. Tight binding and a direct path of retinol from RBP through STRA6 via CRBP1 and LRAT to form RE would seem to preclude atRA formation. On the other hand, a chaperone might have limited function, if retinol could easily escape its influence. The ability of holo-CRBP1 to serve as substrate for retinol metabolism addresses these issues. This concept has sometimes been misunderstood as suggesting that select enzymes require holo-CRBP1 to function, or “engulf” holo-CRBP1 as a substrate, rather than the existence of a microenvironment in which holo-CRBP1 interacts with enzymes in context of their membranes (LRAT and RDH). This would allow transfer of retinol from CRBP1 to select

enzymes without requiring diffusion throughout the cell. In other words, select enzymes participate in a microenvironment with holo-CRBP1 and can "seize" retinol as CRBP1 undergoes the dynamic motions that partially expose but don't release retinol. Put another way, the chaperone model postulates that CRBP1 safeguards *efficient* retinol metabolism; not that retinol metabolism has an absolute requirement for CRBP1. Reactions that occur in the presence of a chaperone, also may occur in the absence of a chaperone. In the absence of a chaperone, however, additional reactions may occur, which normally are restricted by the chaperone. Evidence for this mechanism has been presented in detail [40, 41].

CRBP1-null mice reproduce normally, show no obvious morphological abnormalities, and show no obvious signs of gross atRA deficiency [42]. Hepatic stellate cells in *CRBP1*-null mice, however, have fewer and smaller lipid droplets than WT after 4 weeks of age. Liver has ~50% lower liver retinol and RE concentrations, when fed a chow diet (i.e. copious vitamin A). Low liver RE levels reflect (in part?) a 6-fold faster elimination rate. atRA concentrations in serum and several target tissues do not differ significantly between *CRBP1*-null and WT mice, explaining lack of obvious gross abnormalities [43, 44]. These observations are consistent with CRBP1 functioning as a chaperone that conserves retinol by restricting access to retinol. In the absence of CRBP1, RDH would still convert retinol into retinal for atRA biosynthesis, but additional enzymes also would have access to retinol, prompting the drain on RE. Whether lack of CRBP1 also impairs RE biosynthesis has not been tested. Remarkably, thorough analyses of RE, retinol, retinal and atRA concentrations across the spectrum of retinoid target tissues have not been reported in the *CRBP1*-null mouse. Physiological effects of CRBP1 remain to be elucidated fully, especially concerning more subtle and/or long term effects on retinoid-supported processes.

5. Retinol dehydrogenases

The first of two dehydrogenations in the path of atRA biosynthesis, the conversion of retinol into retinal, is rate-limiting (lowest V_m values) in the presence of CRBP1 [45, 46]. Similar to RE formation, the rate of retinal biosynthesis from holo-CRBP1, catalyzed by microsomal members of the short-chain dehydrogenase/reductase (SDR) gene family, exceeds that allowed by the amount of free retinol in equilibrium with holo-CRBP1; and a Michaelis-Menten relationship occurs between holo-CRBP1 and microsome-catalyzed retinal biosynthesis [47]. Varying the ratio apo-CRBP1/ holo-CRBP1 (within limits), which changes the percent of unbound retinol severely, but does not affect the percent of retinol bound measurably, does not change the K_m value or rate of retinal production from holo-CRBP1, catalyzed by microsomes. This also indicates involvement of holo-CRBP1 itself. Notably, the K_m values of LRAT (0.2 μM) and RDH1 (~2 μM) for holo-CRBP1 are less than their K_m values for unbound retinol of 0.6 and 4 μM, respectively. The points remain, each reaction rate (esterification and dehydrogenation) catalyzed by microsomes in the presence of holo-CRBP1 exceeds that supported by unbound retinol, is saturable, and shows a higher affinity than for unbound retinol. Additionally, N-ethylmaleimide, which binds covalently to sulfhydryl groups in RDH, causes 90% inhibition of holo-CRBP1 supported generation of retinal, but does not inhibit retinal synthesis from unbound retinol [48]. This indicates that N-ethylmaleimide does not affect the catalytic action of RDH1, but interferes with its approach to holo-CRBP1, interrupting retinol transfer. A substrate-enzyme relationship between holo-CRBP1 and RDH16, the human ortholog of mouse RDH1 (rat RDH2) has been confirmed [49, 50].

Retinol and other retinoids are promiscuous substrates, making it difficult to study atRA biogenesis in vitro from unbound retinol. These difficulties are exacerbated by the ability of aqueous media and/or reduced pyridine nucleotides to oxidize retinol or reduce retinal in the absence of enzymes, and contamination by commercial retinol with several percent retinal.

Presenting retinol bound to CRBP1 (as holo-CRBP1) potentially avoids these problems because of its high affinity for retinol.

Studies of atRA biosynthesis focused by use of holo-CRBP1 as substrate showed that 80 to 94% of cellular retinal-generating capacity resides in microsomes, rather than cytosol, and microsomal rates in various tissues exceed cytosolic rates by 5 to 20-fold [51, 52]. Recombinant holo-CRBP1, covalently modified with a cleavable light-activated cross-linking reagent, was used as bait to identify potential RDH. This construct identified two acceptors specifically in microsomes (which obviously contain hundreds of proteins), a 25 kDa band, now known to be LRAT, and a 35 kDa band subsequently identified as an SDR family member [53]. Notably, crosslinking required the presence of a pyridine nucleotide cofactor (NAD^+ or $NADP^+$) and occurred only with holo-CRBP1, not with apo-CRBP1. This is significant because dehydrogenases, such as SDR, function via an ordered bisubstrate reaction mechanism, i.e. they must bind cofactor (aka cosubstrate) *before* they can bind the substrate to be oxidized. Thus, not only did the SDR bind holo-CRBP1 and not apo-CRBP1, it recognized holo-CRBP1 as expected for a substrate. This reinforced the observations noted above with respect to the Michaelis-Menten kinetics between microsomes and holo-CRBP1 for retinal generation. This information led to the first cDNA cloning of an RDH in the path of atRA biosynthesis, rat RODH1 [54]. Subsequently, the human and mouse orthologs were cloned, based on the data generated with the rat cDNA [50, 55, 56]. RDH1 (the mouse ortholog of rat RODH1) contributes to a reconstituted pathway of atRA biosynthesis, when expressed in intact cells with each of the three RALDH. These data set the stage for cloning a large sub-family of SDR associated with retinoid metabolism, reviewed in [57]. Currently, at least three RDH seem physiologically involved in converting all-*trans*-retinol into all-*trans*-retinal: RDH1, RDH10 and DHRS9 (Table 1). All are microsomal members of the SDR gene family; several retinal reductases, RRD, also have been identified. The three RDH have emerged as the primary catalysts of the first step in atRA generation from retinol, because when ablated, each demonstrates a phenotype directly associated with atRA function. It is reasonable, however, to suspect that others may emerge.

Not all agree that RDH interact with/or recognize holo-CRBP1 [58]. Rather, another model postulates that RDH recognize only unbound retinol as substrate. This group revised their original conclusion based on reexamination of RDH16 (human ortholog of RDH1) kinetics that showed a lower rate of retinal production from holo-CRBP1 relative to unbound retinol. This argument suffers from the fact that RDH in vivo would not have access to the relatively high amounts of unbound (free) retinol substrate that were provided in vitro. In vivo, any putative retinol dehydrogenase would have to complete with apo-CRBP1 for retinol: therefore, a high catalytic rate in vitro in the absence of holo-CRBP1 cannot predict performance in CRBP1-expressing cells.

The revised conclusion also was based on RDH16 over expressed in insect cells, incorporated into proteoliposomes, and then reacted with an N-terminal fusion of CRBP1 to glutathione S-transferase or with a C-terminal fusion of CRBP1 to the chitin binding domain. A key aspect of interpreting these data is the catalytic constant (turnover number), k_{cat}, of the RDH16 used. The k_{cat} denotes the number of moles of product produced *per sec* (to keep the numbers manageably low) by one mole of enzyme. The RDH16 used had a k_{cat} of 1.1 min^{-1}, i.e. this formulation of RDH16 produced only one mole of product per mole of enzyme in 50 sec, indicating an enzyme preparation with severely impaired activity. As it is, endogenous RDH1 in its native mammalian environment (microsomes in vitro) generates retinal from holo-CRBP1 at a much lower rate than it does from unbound retinol, possibly because release of retinol from the very high-affinity holo-CRBP1 imposes a rate-limiting step relative to the catalytic cycle of RDH1/16 [47, 52, 59]. The appropriate issue involves

whether the rate catalyzed by native RDH1 from holo-CRBP1 can sustain the atRA concentration in cells—which it can. A preparation with impaired activity, such as the Sf9 generated and solubilized enzyme, may not appear to interact with holo-CRBP1 because of its already low activity, or may not interact because the changes that impaired activity impair interactions. Use of modified CRBP1 also is problematic. Mutating a single external residue (L35) of CRBP1 reduced significantly the V_m for generating retinal from endogenous microsomal RDH1 and increased the K_m value, but did not change the affinity for retinol binding (apparent k_d value) [60]. This suggests that altering the CRBP1 structure, as amending it with peptides at either end, alters its ability to function.

A conclusion that RDH10 does not access holo-CRBP1 as substrate also was based on a significantly lower reaction rate with holo-CRBP1 vs. free retinol, and just as for RDH16, no test was conducted of a possible Michaelis-Menten relationship between RDH10 and holo-CRBP1 [61]. In this case, a modified RDH10 (RDH10-His_6) was expressed as 10% of total insect (Sf9) cell membrane protein—recall that modifying RDH with N-ethylmaleimide inhibited holo-CRBP1-supported generation of retinal by 90%. Nor did the formulation of RDH10 duplicate the microenvironment of native RDH10 associated with mammalian membranes. Membrane-associated proteins depend on membrane composition and their stoichiometry with membrane phospholipids/proteins for native activity and function [62-64]. It is reasonable to expect that the composition and stoichiometry of smooth endoplasmic reticulum membrane phospholipids are crucial to RDH activity and any interaction with holo-CRBP1. For example, RDH1 activity during purification was maintained only with phosphotidylcholine—no other phospholipid tested produced the same reaction rate—and only when RDH1 was bound with the membrane protein, CYP2D [48, 53, 65]. In other words, the membrane context of RDH affects function.

A model in which RDH would recognize only unbound retinol relies on competition between RDH and apo-CRBP1 for retinol (Figure 3A). As stated above, the k_d value of apo-CRBP1 for retinol likely does not exceed 0.1 nM [38, 58]. In contrast, K_m values recorded for RDH with unbound retinol vary from 1 to 6 μM—with holo-CRBP1 as substrate the K_m values vary from ~0.6 to 2 μM—but in each case the values are lower than those values generated in the same report for unbound retinol [54-56, 66]. RDH10, which reportedly has a K_m value of 0.035 μM for unbound retinol, may be an exception [61]. But this value was obtained with a substrate concentration range of 0.0625 to 2 μM. In other words, it is unreliable because the lowest substrate concentration tested was much higher than the inflection point of the curve; and the value was generated with RDH10-$(His)_6$ expressed as 10% of Sf9 cell microsomal protein. In contrast, native human RDH10 assayed in membranes of COS-1 cells had an K_m value of ~4 μM for all-*trans*-retinol [67]. To be sure, K_m values do not consist only of k_a and k_d values of enzymes for their substrates, but K_m values reflect association/dissociation constants to a large extent. This model would have two proteins competing for retinol, which differed by as much as 40,000-fold in affinity for retinol (0.1 nM k_d vs. ~4 μM K_m) (Figure 3B). Although this is a “back-of-the-envelope” calculation, it illustrates the issue. The lower affinity protein, in this case RDH, would have little chance of sequestering substrate (retinol). In the presence of CRBP1, the steady-state concentration of retinol would be nM, whereas the steady-state concentration of holo-CRBP1 would be μM; making holo-CRBP1 the highest concentration potential substrate (Figure 3C). In effect, RDH with μM K_m values would have available low nM retinol, likely associated with membranes, or μM holo-CRBP1. Consider this with coupled retinol uptake and esterification, and it seems that generation of retinal would depend on the close relationship between holo-CRBP1 and RDH (indicated by the cross-linking that occurred only under conditions that support the catalytic cycle of RDH). Ability of CRBP1 to bind retinal, as well as retinol, and ability of RALDH1 and 2 (RALDH3 has not been tested) to

generate atRA from CRBP1-retinal complete the argument for a process that does not rely on unfettered diffusion of retinol away from CRBP1.

6. An N-terminal leader anchors RDH and RRD in the ER facing cytoplasm

The orientation of RDH1 and similar SDR in the endoplasmic reticulum (cytoplasmic or lumenal facing) has been determined by multiple approaches [68-70]. The properties of native RDH1 were compared to a chimera with an established luminal signaling sequence, 11β-hydroxysteroid dehydrogenase(aa1-41)-RDH1(aa23-317). In addition, the green fluorescent protein fusions RDH1(1-22)-GFP and 11β-HSD1(1-41)-GFP and signaling sequence mutants were examined via confocal immunofluorescence, antibody access, proteinase K sensitivity, and deglycosylation assays [69]. The results showed clearly that an N-terminal signaling sequence of 22 residues anchors RDH1 in the endoplasmic reticulum projecting into the cytoplasm. Deleting the positive charges from the C-terminal end of the leader, and inserting two arginine residues near the N-terminus of the signaling sequence caused 95% inversion from cytoplasmic to luminal: i.e. the mutant L3R,L5R,R16Q,R19Q,R21Q faced the lumen. A retinal reductase, RDH11, with an N-terminal signaling sequence similar to that of RDH1, also orients to the cytosol, as indicated by a variety of approaches, including deglycosylation assays and mutagenesis [70]. In this case, in contrast to the native protein, the R25G,K26I mutant showed additional bands on SDS-PAGE, which were eliminated by Endo H treatment, i.e. the mutant was flipped partially from a cytosolic to a lumenal orientation by eliminating the positive charges at the C-terminal end of the signaling sequence. These results are consistent with the lack of RDH1 and RDH11 glycosylation, in contrast to lumenal facing proteins. These conclusions also are consistent with the “positive-inside rule”, which reflects the observation that positively-charged residues in leader sequences are 4-fold more abundant on the cytoplasmic side of membrane proteins, i.e. positive residues at the C-termini of leader sequences orient membrane proteins to the cytosol [71]. A refined version of the positive-inside rule takes into account the net electrical charge difference, as well as the hydrophobicity of the leader. By these criteria, the experimental data and the amino acid composition of the leaders in RDH and RRD indicate orientation to the cytosol. Alternative work concluded that the mouse RDH4 (human RDH5) orients to the lumen [72]. This conclusion was based on limited data (relative to the studies discussed above) with a mutant, and did not address effectively the extensive data discussed above, including: 1) how RDH/RRD manage to escape glycosylation, even though they harbor prototypical glycosylation signals (and lumenal proteins are glycosylated); and 2) how mutating the positive charges in the leaders of RDH/RRD allowed glycosylation, i.e. “flipped” the enzymes. Finally, a lumenal orientation for RDH4/5 would seem to preclude function as an 11-*cis*-retinol dehydrogenase to generate 11-*cis*-retinal for binding to opsin.

7. Xanthine oxidase

The cytosolic enzyme xanthine oxidase (aka xanthine dehydrogenase) generates atRA from retinol (through retinal) in human mammary epithelial cells [73, 74]. The reaction depends “strictly” on CRBP1 and has a K_m value for holo-CRBP1 of ~0.1 μM. This is an intriguing observation, and may represent the holo-CRBP1 recognizing activity in cytosol (recall the discussion above that 6 to 20% of cellular retinal-generating capacity occurs in cytosol), but awaits further study to confirm its contribution to atRA biosynthesis.

8. Retinal dehydrogenases

The next step in atRA biosynthesis involves a rapid (relative to retinol dehydrogenation) and irreversible dehydrogenation of retinal into atRA. At least three RALDH catalyze this step. As with identification of the first step enzymes, retinoid binding-proteins contributed to

identification of RALDH candidates as physiological participants in atRA biosynthesis. Lee et al. showed that two mouse ALDH, AHD-2 and AHD-7, converted unbound retinal into atRA in vitro with K_m values <1 μM [75]. Neither seemed to have widespread tissue distribution, however, prompting concern about their universal importance in atRA generation. Also, at the time, the mouse was thought to express at least 13 ALDH, but the rat was thought to express only four, not including AHD-2 [76]. Rather, the closest ALDH to AHD-2 in rat was known as phenobarbital-induced ALDH [77]. Apparent absence of an ortholog in rat liver argued against the central importance of AHD-2 to atRA biosynthesis. Fractionation of rat cytosol, however, revealed multiple RALDH activities with unbound retinal. To determine which might participate physiologically in atRA biosynthesis, retinal generated from holo-CRBP1 by microsomes was used as bait (CRBP1 binds retinal, not only retinol), and an enzyme was identified that was regulated by vitamin A status and atRA [78]. This resulted in purification of rat liver cytosolic RALDH1 as the ortholog of AHD-2 and the demonstrations that RALDH1 differs from phenobarbital-induced ALDH, is down regulated by vitamin A in liver, and is inhibited by apo-CRBP1. Subsequently, the importance of AHD-2 (RALDH1) to atRA biosynthesis was demonstrated in the dorsoventral axis of the mouse retina [79]. Further work purified RALDH1 from rat kidney [80]. Working from the amino acid sequence of RALDH1, RALDH1 was cloned and the enzyme was characterized as an RALDH with widespread tissue distribution and tissue-specific regulation by atRA [81-83]. RALDH1 recognizes CRBP1-bound retinal as substrate ($K_{0.5}$ = 0.8 μM) and is inhibited by apo-CRBP1, with an IC_{50} of 1.4 μM.

RALDH2 was first cloned from rat testis using information from RALDH1 [84] and was cloned subsequently from P19 cells [85]. RALDH2 enjoys widespread tissue expression, often overlapping with RALDH1, but is regulated differently than RALDH1 by vitamin A status, and is not inhibited by apo-CRBP1 [86], although RALDH2 recognizes CRBP1-bound retinal as substrate with a K_m value of 0.2 μM. (This differential response to apo-CRBP1 suggests RALDH1 and 2 operate during differing degrees of vitamin A status, and illustrates the complexity of atRA biosynthesis.) Like RALDH1, RALDH2 can generate atRA from retinal biosynthesized by microsomes from holo-CRBP1, and can function in a reconstituted atRA “generating system” in intact cells co-transfected with RDH1. A third RALDH, RALDH3, was later identified and originally referred to as ALDH6 [87].

RALDH1, RALDH2 and RALDH3 exhibit distinct expression patterns during mouse development, indicative of locus and stage specific individual functions for each [88].

A fourth RALDH, RALDH4 has been cloned, but RALDH4 recognizes only 9-*cis*-retinal as substrate and not all-*trans*-retinal [89]. Unlike RALDH1, 2 and 3, the contribution of RALDH4 to retinoid metabolism remains unexplored.

9. Disposition of atRA

atRA associates with binding proteins of the FABP gene family: CRABP1, CRABP2 and FABP5 [90, 91]. Holo-CRABPII interacts with and transfers atRA to RAR [92-95]. In contrast, FABP5 transfers its atRA to PPARβ/δ [96-98]. Thus, a mechanism that differentiates various atRA actions seems to be selective delivery to specific nuclear receptors mediated by binding to CRABP2 vs. FABP5 [99, 100].

The total physiological function of atRA binding to CRABP1 has not been resolved, but microsomes can access atRA bound with CRABP1 and generate catabolites with a lower K_m value and higher rate than for unbound atRA, suggesting that one function of CRABP1 may include modulating atRA concentrations [101]. Consistent with this hypothesis, over expression of CRABP1 reduces atRA potency to induce F9 cell differentiation and increases its rate of catabolism [102-104].

atRA catabolites include: 5,6-epoxy-atRA, 4-oxo-atRA, 4-hydroxy-atRA, and 18-hydroxy-atRA [105-112]. Three cytochrome P-450 gene family members with widespread tissue distribution have been established as primary catalysts of atRA degradation, CYP26A1, B1 and C1[112-118]. The mouse expresses a fourth, CYP2C39, but the rat and the human do not express this P-450 [119]. Additional CYP also catabolize atRA in liver: including the rat CYP22C and the human CYP22C8 (and perhaps the human CYP22C9) [115, 120].

10. Regulation of atRA homeostasis

Biosynthesis of RA is the only established function of retinol, outside of generating retinal in the eye. Thus, it makes sense that atRA regulates retinol concentrations and autoregulates its own concentrations. CRBP1 and/or LRAT are essential for hepatic buildup of retinol and RE, and affect tissue distribution of retinol [42, 121-124]. As mentioned above, “excess” retinol is diverted into RE. Retinol cycles between its non-esterified and esterified forms, seemingly with the ratio apo-CRBP1/holo-CRBP1 contributing to the direction of flux. atRA induces *RBP1* (encodes CRBP1) and *LRAT* gene expression [8, 125, 126]. Induction of *RBP1* expression by atRA may represent a “housekeeping” function, as the amount of CRBP1 does not seem to vary extensively with variations in vitamin A status.

Challenging normal human keratinocytes in culture with increasing retinol did not substantially increase cellular retinol or atRA concentrations with a wide retinol concentration range [127]. Retinol and atRA remained low because of retinol storage via LRAT as RE. In vivo, an increase from a diet with 25 IU/g vitamin A (copious) to a very high vitamin A diet (250 IU/g), results in a 5-fold increase in liver retinol (to 0.3 μmol/g) and ~2-fold increase in RE to the very high concentration of ~7.9 μmol/g [123]. Lung, rather than adipose, stores the second highest concentration of RE in mice fed the copious diet (~1.6 μmol/g), and the greatest concentration of RE in mice fed the very high vitamin A diet (~11 μmol/g). In other words, organisms have very high capacity for storing vitamin A as RE—a substrate driven process with retinol uptake linked to RE formation. The *LRAT*-null mouse has very much lower tissue concentrations of retinol and RE than WT when fed both a copious and very high vitamin A diet, except for adipose retinol (with retinol variable in kidney), and maintains retinoid homeostasis by increased gastrointestinal excretion (very high diet only), redistribution of retinol to adipose (both diets), and increased retinoid catabolism via induction of liver CYP26A1 expression (both diets). These data reveal why loss of LRAT does not produce unbridled atRA biosynthesis: retinol decreases in most tissues and the increase in adipose is not sufficient to drive extraordinary atRA biosynthesis and/or compensatory mechanisms (CYP induction?) accompany loss of LRAT or CRBP1.

LRAT expression reflects dietary vitamin A, but does not show a wide range of expression in the marginal to ample dietary vitamin A range, suggesting that atRA has a “housekeeping” function to maintain LRAT expression [128]. The transition from a vitamin A-deficient diet to a diet with marginal vitamin A (1.3 IU/g) causes a 9-fold induction in LRAT, but an increase from the marginal diet to one that contained ample vitamin A (i.e. 13 IU/g) did not change LRAT expression substantially in rat liver. An increase to a very large amount of dietary vitamin A (333 IU/g) induced LRAT only 2.2-fold relative to the diet with marginal vitamin A. These data are consistent with the retinoid storage data mentioned in the previous paragraph.

The *CRBP1* and *LRAT* knockout data show that lab mice under carefully controlled conditions do not require CRBP1 or LRAT for survival. Obviously, both are essential in the wild, because both are highly conserved, and wild mice do not enjoy the living conditions of lab mice.

atRA also induces *STRA6* mRNA [129, 130]. This simultaneous regulation of *STRA6*, *CRBP1*, and *LRAT* reinforces the concept discussed above of coupled retinol uptake and esterification, with "excess" retinol directed into RE, rather than to large amounts of non-esterified retinol. atRA treatment in association with retinol causes maximum accumulation of RE, reflecting substrate-driven esterification during atRA-induced CRBP1 and LRAT expression.

Another aspect of autoregulating atRA concentrations involves induction of *CYP*, which increases the rate of atRA catabolism [131-133]. Increasing dietary vitamin A from marginal (0.6 IU/g diet) to the AIN recommended amount (4 IU/g diet) induced mouse liver CYP26A1 mRNA 8-fold, but produced no change in CYP26B1 [3]. Feeding mice a diet with copious vitamin A (30 IU/g) induced mouse liver CYP26A1 >60-fold relative to the 4 IU/g diet and 500-fold relative to the 0.6 IU/g diet. A diet with copious vitamin A also induced liver *CYP26B1* ~10-fold relative to the 4 IU vitamin A/g diet. In the rat, a diet with marginal vitamin A increases *CYP26A1*, *CYP26B1* and *CYP2C22* mRNA ~5-fold, ~2-fold, and ~23-fold, respectively, relative to a vitamin A-deficient diet [128]. Progressing from the diet with marginal vitamin A to one with an ample amount of vitamin A (13 IU/g) had no further major effects on *CYP26A1* and *CYP2C22* mRNA, but induced CYP1B1 2-3-fold. An increase to a very large amount of dietary vitamin A (333 IU/g) induced *CYP26A1* 20-fold relative to the diet with ample vitamin A, but induced *CYP26B1* and *CYP2C22* only ~3-fold. These data show that atRA regulates its own concentrations through lowering substrate (retinol conversion into RE), and enhancing its catabolism.

Interactions between RDH and RALDH provide another level of maintaining atRA concentrations. In astrocytes, knocking down *DHRS9* results in increased atRA production, despite the fact that DHRS9 functions as a retinol dehydrogenase in intact cells, not as a reductase [134]. This resulted from an increase in *RALDH1* expression.

11. Possible functions for multiple RDH and RALDH

Occurrence of multiple RDH and RALDH, often in the same cells, poses the question, why so many? One might argue that the importance of atRA demands redundancy. On the other hand, the *RDH10* and the *RALDH2* knockouts each are embryonic lethal ~e10.5. These observations argue against only redundancy as a reason for multiple RDH and RALDH, at least under the specific experimental conditions. An alternative hypothesis has each RDH and RALDH generating atRA for a specific function, i.e. distinct intracellular pools of atRA may exist, either with respect to locality and/or in response to distinct signals. Each pool would fulfill a distinct vitamin A function. This is reminiscent of malonyl-CoA, generated by two different isozymes of acetyl-CoA carboxylase (ACC) [135]. In liver cytosol ACC1 generates malonyl-CoA as substrate for de novo fatty acid biosynthesis. In the liver outer mitochondrial membrane, ACC2 generates malonyl-CoA to inhibit carnithine-palmitoyl acyltransferase 1, which prevents acyl-CoA transport into mitochondria to support β-oxidation. The relevant point is generation of the same chemical by two isozymes at distinct subcellular sites in the same cell to serve different functions. The distinct atRA-related phenotypes that result from ablating each of *RDH1*, *RDH10*, *DHRS9*, *RALDH1*, *Radlh2*, and *RALDH3* support such a hypothesis. These distinct phenotypes suggest that each of these enzymes fulfill a specific function that cannot be supported by the others. The ability of each of the three RDH and the three RALDH to function intracellularly has been demonstrated [134]. Primary hippocampus astrocytes express each of the six and each contributes to atRA biosynthesis, as demonstrated by sequential siRNA knockdown. In addition, immunocytochemical analysis revealed cytosolic expression of both *RALDH1* and *RALDH2*, as expected from previous work, but substantial nuclear expression of *RALDH1* and perinuclear expression of *RALDH2*. Even though *RDH1* and *RDH10* also are both expressed

in the smooth endoplasmic reticulum, confocal immunofluorescence reveals their precise expression loci differ.[3]

12. Quantification of physiological amounts of retinoids

atRA is very difficult to detect, let alone quantify, because of its very low abundance, the small sizes of many of the loci it affects (hippocampus, limb buds, e.g.) and its liability. LC/MS/MS assays have been developed to quantify atRA in biological samples [136]. Most have been developed for use with relatively abundant and easy to manipulate matrices (serum, liver, and cells in culture), whereas other assays adapt to a wider range of samples, including samples difficult to assay such as brain sections, adipose, pancreas, and embryo sections. LC/MS quantification of atRA offers sensitive, specific and real-time atRA quantification via analytically robust methodology, if applied rigorously. Reference retinoid values are provided (Table 2). The values for RE, retinol and retinal were generated by high-performance liquid chromatography, as these retinoids are sufficiently abundant to allow quantification by UV detection.

An RARE-lacZ reporter has been used to designate areas of atRA presence, even though it requires tissue-specific transcription cofactors obligatory for activating RARβ. Thus, the design of the reporter limits its effectiveness to those loci that express the necessary cofactors in the required stoichiometry. In addition, the RARE-lacZ reporter lacks sensitivity (false negatives), does not provide quantitative data, is not specific for atRA (false positives), and has not been validated as an assay. Such validation requires (minimally) generation of standard curves, demonstration of intra- and inter- assay reproducibility, insight into specificity, and knowledge of lower limits of detection. Moreover, the RARE-lacZ reporter requires 12 to 14 hr to produce a signal, and produces a signal whose half-life has not been established, which alone would severely limit its use it in developmental studies. For example, analysis of data generated by the RARE-lacZ reporter in the dorsal forebrain of the *Foxc1* hypomorph revealed the signal mostly persisted from activation at a developmental stage earlier than the one studied [137]. These authors compared LC/MS data to RARE-lacZ reporter data, and concluded that the latter was “not useful” in assessing function of atRA in the developing cortex.

13. Retinoid-related processes served by RDH

RDH1

Although the mouse embryo expresses *RDH1* as early as e7.5, and the mouse, rat and human orthologs have widespread tissue distribution, the *RDH1*-null mouse shows no obvious developmental phenotype [3, 54-56, 66]. Decreases in expression of CYP that degrade atRA and increases in other RDH and RALDH may account for survival of the *RDH1*-null mouse. Some developmental biologists have concluded that this shows RDH1 is not “essential” for development. This is understandable, concerning a morphological view of development. On the other hand, the concept that a well-conserved gene expressed early and widely (species and tissue) is not “essential” for development seems contradictory to the concept of natural selection, especially when its expression increases 40-fold between e7.5 and e18.5 and drops precipitously at P2. The ablated enzyme might cause (partial) cell function failure that could contribute to serious diseases that develop over a lifetime, such as type II diabetes, even though they do not produce a morphologically defective embryo. Another issue is the artificial environment of laboratory mice. Laboratory mice are products of unnatural selection and are larger, fatter and quicker to mature than mice in the wild [138-141]. Laboratory mice have larger litters, and are slower, weaker, and less active than wild mice.

[3]Weiya Jiang and J. Napoli, unpublished data.

In addition, mouse chow contains copious amounts of nutrients that exceed even the generous amounts recommended by the AIN for rodent chow, which does affect the outcome of gene deletion experiments [15].

A chow diet contains copious vitamin A (15 to 30 IU/g, depending on the formula), which presumably overwhelms retinoid binding-proteins and allows generation of atRA by enzymes that normally would not have access to retinol. In contrast to a lack of phenotype when fed a chow diet, when fed a vitamin A-deficient diet (note: feeding a vitamin A-deficient diet to a mouse bred from a dam fed a chow diet does not induce vitamin A-deficiency up to 40 weeks), or the AIN recommended amount of vitamin A , the *RDH1*-null mouse gains weight faster than WT starting after weaning, and becomes up to 30% fatter than WT controls. This is remarkable, as most other models of obesity require feeding a high-fat diet for many months, even to genetically altered mice. Prevention of the phenotype by a chow diet emphasizes the ability of excess vitamin A to rescue the phenotype and the pitfalls of using chow diets to investigate knockouts related to vitamin A action. The precise mechanism of the phenotype remains unresolved, but the highly specific phenotype reinforces the hypothesis that each of the multiple RDH generate atRA to serve specific functions.

atRA protects against nephropathy in HIV-1 transgenic mice (Tg26 mice). Conversely, atRA was reduced 50% in the kidney cortex of Tg26 mice and 70% in their glomeruli [142]. This was attributed to a 70% reduction in *RDH1* mRNA and ~2-fold increase in *CYP26A1* mRNA. These data indicate that a defect in atRA synthesis contributes to the pathology of HIV in kidney and illustrates a physiological function for RDH1 in generating atRA.

RDH10

RDH10 was first cloned and evaluated kinetically as a retinol dehydrogenase in the retinal pigment epithelium, and later was found in Muller cells [143, 144]. RDH10 was subsequently correlated with several sites of atRA biosynthesis in the mouse embryo, suggesting functions outside of the eye [145, 146]. Like, RDH1, RDH10 is active with both all-*trans*-retinol and 9-*cis*-retinol [61].

RDH10 in conjunction with RALDH2 generates atRA in human dendritic cells [147]. The PPARγ agonist, rosiglitazone, induced the mRNA of RDH10 and RALDH2, followed by induction of atRA biosynthesis. The atRA generated, functioning via RARα induced CD1d, a receptor for xenobiotic lipids. NKT cell development requires CD1d and Cd1d regulates T-lymphocyte function [148]. These data demonstrate contributions for RDH10 and RALDH2 in generating atRA for immune system performance.

ENU-mutagenesis generated a point mutation that impaired embryogenesis, which has been traced to loss of RDH10 activity [149]. A single amino acid substitution destabilizes RDH10 and eliminates its activity. This impact on retinol dehydrogenase activity is consistent with the demonstration that a single mutation in RDH1 causes a 1250-fold decrease in activity [150]. The mutations in both cases were not within the cofactor binding domain or in active site residues. The RDH10 mutant produced defects at e10.5 in limbs, optic vesicles and caudal pharyngeal arches, and morphological defects suggestive of neural crest cell defects. Mice died by e13.5. Significantly, an admittedly “imperfect” reporter, i.e. an RARE-lacZ transgene, detected residual atRA biosynthetic activity in these mutants. If the reporter data are taken at face value, these data suggest contributions of other RDH to embryogenesis, as does the fact that RDH10 mutants did not suffer defects in all atRA-directed processes during embryogenesis.

A more comprehensive study tied developmental abnormalities of *Foxc1* hypomorphs to decreased atRA signaling resulting from decreased *RDH10* and *RALDH2* expression [137]. *Foxc1* hypomorphs lack dorsal forebrain meninges, which relate to decreases in intermediate progenitor cells and neurons by e14.5, resulting in cortical abnormalities. This suggested a diffusible factor that directs progression of progenitor cells, which was identified as atRA. atRA generation was related to RDH10 and RALDH2, which had substantial, but not complete expression loci overlap, again suggesting contributions from other RDH and/or RALDH to atRA biogenesis in the embryo. This study then examined an *RDH10* hypomorph viable until e16.5 and noted that the dorsal forebrain defects were similar to those of the *Foxc1* hypomorph, as was a reduction in cortical neurons. Reductions in atRA were not total, but rather approached 50% in the cortices (quantified by LC/MS), again indicating alternative sources of atRA. These data support the conclusions that atRA functions as a meningeal-derived signal for corticogenesis, and RDH10 contributes significantly to generating the atRA, but isn't the only RDH that contributes to the path of atRA biosynthesis during embryogenesis.

DHRS9

DHRS9 has been cloned multiple times resulting in several designations. DHRS9 recognizes both free- and CRBP1-bound retinol as substrate [50, 59]. Additional data that DHRS9 functions in the physiological generation of atRA were developed in the zebrafish, which require DHRS9 for normal gut development and differentiation [151]. Zebrafish express *DHRS9* ubiquitously in early embryogenesis, but occurs only in the gut 3 days after fertilization. *DHRS9* knockdown at the one-cell stage produced a phenotype revealing a lack of atRA-directed embryogenesis, which was rescued by dosing with atRA. In addition, adenomatous polyposis coli mutant zebrafish turn out as hypomorphs in *DHRS9* expression: these mutants also are rescued by dosing with atRA, or by replacing DHRS9.

Human colon and epidermis in the atRA–dependent strata epidermis express *DHRS9* most intensely. Interestingly, relative to normal tissue, human colon adenoma and carcinoma samples showed reduced expression of *DHRS9* and *RDH5*—but the latter SDR is better known for its activity in the retinal pigment epithelium with 11-*cis*-retinol [152]. Colon carcinoma cell lines, which had low rates of atRA biosynthesis from retinol, albeit relative to normal human mammary epithelia cells, did not express either *DHRS9* or *RDH5*. Introduction of the tumor suppressor adenomatous polyposis coli into the colon carcinoma cell line HT29 induced *DHRS9* 5-fold (qPCR) via the transcription factor Cdx2, but did not induce *RDH5*, and increased atRA biosynthesis about 2-fold. Consistent with a function of DHRS9 in generating atRA to suppress tumorigenesis, screening of 19 tumor cell lines found that the Burkett lymphoma-derived cell line DAUDI does not express *DHRS9*. Similar observations were made with the MOLT-14 cell line (derived from Burkett lymphoma) and the T-cell acute lymphoblastic leukemia cell line Raji [153].

DHRS9 also seems important to atRA function during the estrus cycle. The rat uterine lining epithelium expresses *DHRS9* only during estrus and co-expresses the SDR with *CRBP1* and *CRABP2* [154] and estrogen induces *DHRS9* expression [155].

DHRS9, however, has not been knocked out in mice to understand its precise contribution to atRA biogenesis in the intact animal. Yet, its clear association with atRA supported processes render it an "RDH of interest". Recalling the evidence that RDH1 and RDH10 are not capable of fulfilling all needs for atRA in the embryo, a function for DHRS9 in atRA biogenesis seems likely.

14. Visual cycle RDH

Multiple RDH/SDR function in the visual cycle to convert 11-*cis*-retinol into 11-*cis*-retinal in the retinal pigment epithelium, and to reduce all-*trans*-retinal into all-*trans*-retinol in photoreceptor outer segments [156]. Although, the precise RDH involved in each step remain a matter of discussion, RDH5, RDH10 and RDH11 seem to contribute to 11-*cis*-retinal production, whereas RDH8, RDH12 and retSDR1 reduce all-*trans*-retinal [157-161].

15. Retinoid-related processes served by RALDH (Aldh1A)

RALDH1 (Aldh1A1)

RALDH1-null mice are fertile and indistinguishable from WT littermates [162]. In the adult, RALDH1 accounts for ~90% of the all-*trans*-retinal dehydrogenase activity in kidney and liver of rat and mouse, and contributes substantially to atRA biosynthesis in many other tissues [75, 163-165]. RALDH1 has been associated with obesity in the adult. *RALDH1* expression is up-regulated 3-fold in the kidney of *db/db* mice, whereas atRA concentrations are lower, consistent with regulation feedback regulation of *RALDH1* by atRA [82, 166]. *RALDH1*-null mice resist glucose intolerance and obesity when fed a high-fat diet [167]. This observation triggered a hypothesis that retinal itself functions as a “distinct transcriptional regulator”, based (in part) on an increase in retinal in fat in knockout mice. But atRA in fat was not measured, and therefore changes in atRA were not excluded as underlying the phenomena. Although dosing with retinal reduced subcutaneous fat as a percentage of total fat in *ob/ob* mice more efficiently than dosing with atRA (15.5% vs. 19.1%), the conclusion that retinal must be functioning *per se* did not consider the distinctive pharmacokinetic characteristics of retinoids, and the metabolic vulnerability of retinal, and also was not supported with atRA measurements. The mechanism proposed, that retinal suppresses PPARγ-mediated processes, seems unlikely because the k_d value of all-*trans*-retinal for PPARγ was reported as ~12 μM, far above the usual k_d values for ligands of nuclear receptors, and the physiological concentration of retinal in white adipose (~64 pmol/g) [34]. A subsequent study of *RALDH1*-null mice addressed the atRA issue, and concluded that RALDH1 generates the major amount of atRA used to promote adipogenesis (relative to RALDH2 and 3), and RALDH1 deficiency impairs atRA production, which decreases atRA signaling, *ZFP423* expression, *PPARγ* expression, and adipogenesis, specifically in visceral fat [168]. These later data provide a viable mechanism for *RALDH1*-null mice resisting weight gain when fed a high-fat diet.

A 56 kDa low-affinity androgen-binding protein, which occurs only in genital skin fibroblasts and not in non-genital fibroblasts, has been identified as RALDH1 [169, 170]. Curiously, *RALDH1* is not expressed in most humans with androgen receptor mutations that cause androgen insensitivity. These mutations lead to testicular feminization and/or reduced fertility. The hypothesis has been suggested that lack of *RALDH1* is causal in these physiological impairments, given the function of RALDH1 in atRA biosynthesis [171, 172].

The *RALDH1*-null mouse develop cataracts by 6-9 months old, indicating a function in protecting the eye other than by generating atRA [173].

Molecular insight into regulation of *RALDH1* expression was generated from a *RALDH1*-CAT construct consisting of a proximal promoter upstream of the transcription start site, evaluated in the mouse cell line Hepa-1. The CCAAT/enhancer-binding protein, C/EBPβ, induced *RALDH1* expression via a CCAAT box, whereas atRA decreased *RALDH1* expression via RARα by decreasing the C/EBPβ concentration [174]. A subsequent study verified the role of the CCAAT-binding protein and added a role for the Oct transcription factor in the kidney, both within −80 and +43 of the 5′-region [175]. This was latter

confirmed and extended by the observation that atRA increases GADD153, which sequesters C/EBPβ [176].

Notably, *RALDH1* is the only RALDH expressed in the adult rat pituitary gland [177]. Estradiol decreases *RALDH1* expression via ERα in the pituitary [178, 179].

Mice fed an ethanol-containing diet for one month had increased hippocampus atRA and RALDH1 activity, despite a decrease in RALDH1 protein and no notable RALDH2 activity or high RALDH3 activity, suggesting post-translational modification of RALDH1 [180]. atRA stimulates protein tyrosine kinase [181], and RALDH1 has been identified as a tyrosine phosphorylated protein in nasopharyngeal tissues [182]. This indicates that RALDH1 may undergo post-translational regulation by atRA, in addition to transcriptional regulation.

Even though there is no observed phenotype for in the embryo of the *RALDH1*-null mouse, RALDH1 denotes cancer stem cells and seems to designate a poor prognosis [183]. In addition, as well as other members of the ALDH gene family, also serve as markers of normal stem cells [184]. For example, the developing mouse and human express *RALDH1* in pancreas at various stages during β-cell generation; atRA induces $Ngn3^{+}$ endocrine progenitor cell formation and stimulates their further differentiation into β-cells [185].

RALDH2 (Aldh1A2)

RALDH2-null mice do not live beyond e10.5. The embryos are malformed and do not undergo the axial rotation that normally occurs on or about e8.5 [186]. Malformations include shortening of the anterioposterior axis, lack of limb buds, a deformed heart, and a truncated frontonasal region. Defects in embryonic rats that model congenital diaphragmatic hernia in humans have been related to inhibiting RALDH2 [187]. Nitrofen, 4-biphenylcarboxylic acid, bisdiamine, and SB-210661 inhibit RALDH2 and produce defects in the rat diaphragm. Interestingly, the atRA-mediated processes that go awry in the *RALDH2*-null mouse differ from those that go awry in the *RDH10* knockout, again suggesting that each enzyme contributes to paths that generate atRA for specific aspects of development, and each may be coupled (partially) with a different RDH or RALDH. The different developmental expression patterns of *RALDH1* and *2* also suggest distinctive biological functions during embryogenesis [188]. These observations have stimulated many studies of *RALDH2* expression during embryogenesis, which are beyond the scope of this review. One conclusion, however, should be emphasized: even though *RALDH2* ablation causes embryonic lethality, RA generating activities occur in embryo loci that are RA dependent and devoid of *RALDH2* expression, including but not limited to *RALDH1* and *RALDH3* [189]. These data show clearly that routes of atRA biosynthesis alternative to RALDH2 occur during all phases of embryonic development.

In contrast to the plethora of studies during embryogenesis, relatively little has been done with RALDH2 postnatally. In the adult rodent, RALDH2 has widespread expression, but is most highly expressed in the testis. Interstitial cells of the testis, spermatogonia, and spermatocytes express *RALDH2* [86, 190]. Restricting dietary vitamin A causes a 70-fold decrease in *RALDH2* mRNA, which reflects massive loss of germ cells, and possibly decreased expression in the remaining cells. *RALDH2* expression often overlaps with that of *RALDH1*, including in hepatocytes and airway epithelia. A so-called “testis-specific aldehyde dehydrogenase” has been confirmed as RALDH2 [191].

atRA modulates nephrogenesis [192]. El Kares et al. have identified a common variant (20% of the human population) in *RALDH2* that was associated with increased cord blood atRA and a 22% increase in kidney volume [193].

RALDH2 mRNA decreases during rat neonate suckling, whereas *RALDH1* mRNA increases during weaning of the rat pup [194]. The mechanisms of these effects have not been established. Estradiol induces *RALDH2*, with most intense induction during metestrus [195]. Promoter analysis indicated an estrogen receptor response element [190]. Subsequent work revealed that estradiol induces *RALDH2* in uterine stromal cells, contrasting with estradiol suppression of *RALDH1* in the glandular epithelium [196]. Gonadotropin also induces *RALDH2* expression intensely. Spinal cord injury induces *RALDH2* in what may be a unique atRA-synthesizing subpopulation of activated oligodendrocyte precursors or "polydendrocytes" [197]. In epicardial cells, *RALDH2* is a transcriptional target of Wt1 [198]. Both TAL1 and LIM-only protein genes, but neither alone, induce *RALDH2* expression when co-transfected into T-cell acute lymphoblastic leukemia cell lines [199]. A GATA site in the second intron binds GATA3 to initiate transcription.

Further analysis of the *RALDH2* promoter indicated the presence of a sterol regulatory element binding protein (SREBP) [190]. In fact, cholesterol induces *RALDH1* and *RALDH2* expression in liver, brain, kidney and heart [200]. Oxysterols mediate the cholesterol effect via SREBP-1c and LXRα/β, establishing that "cross-talk" occurs between atRA biosynthesis and cholesterol metabolism.

RALDH3 (Aldh1A3)

The *RALDH3*-null mouse suffers from choanal atresia, because of persistence of nasal fins at e18.5 [201]. Rupture of nasal fins is a normal development process, which allows communication between oral and nasal cavities. Persistence of the fins causes death from respiratory distress in newborn mice. All malformations of *RALDH3*-null mice are rescued by supplementing dams with atRA during pregnancy—providing a direct link to its physiological importance in generating atRA.

RALDH3 is the only one of RALDH1, 2 and 3 that does not recognize 9-*cis*-retinal as substrate [202].

Whereas the developing mouse and human pancreas express *RALDH1* during β-cell generation, abnormally high pancreas expression of *RALDH3* has been associated with decreased glucose-stimulated insulin secretion in diabetic mouse models [203]. A 9-fold increase in pancreatic islet *RALDH3* mRNA was observed in mice fed a high-fat diet or in *db/db* mice. A high glucose concentration (25 mM) caused a 4-fold induction of *RALDH3*, relative to 5 mM in the rat insulinoma cell line Min6 and a 2-fold increase in the glucagon-producing cell line αTC1. Transfecting an RALDH3 expression vector into Min6 cells caused a 3-fold decrease in insulin secretion, but transfecting αTC1 cells with RALDH3 produced a 2-fold increase in glucagon secretion. The pre*cis*e retinoid responsible for these results was not determined, as only 13-*cis*-RA was investigated, an isomer without known activity. Regardless of the mechanism, these data are consistent with retinoids contributing to pancreas function [204]. These observations, along with different expression patterns of RALDH2 and RALDH3 in the cycling hair follicle of the adult mouse again indicate that each RALDH provides distinct pools of atRA for specialized functions, and that each contributes essentially to adult atRA-mediated functions [205].

RALDH3 seems to generate atRA to maintain differentiation in mammary epithelia, which suppresses tumorigenesis. Primary cultures of normal human mammary epithelial cells convert retinol into atRA, as do the non-tumorigenic mammary epithelial cell lines tested [206]. In contrast, five of six mammary cancer cell lines had either no or vastly diminished ability to biosynthesize atRA. Retinol caused growth inhibition in cells capable of converting retinol into atRA, but not in cells incompetent in atRA biosynthesis. The "missing" enzyme was identified as RALDH3 and re-introduction of RALDH3 into MCF-7

cells, a tumorigenic breast epithelial cell line, reinstituted ability to convert retinal into atRA [207]. In support of this conclusion, Mtsm1 a dominant modifier of mammary tumor susceptibility, has been mapped to a locus with five candidate genes for mammary tumorigenesis, one of which is *RALDH3* [208]. In contrast to these data, RALDH3 has been identified as a cancer stem cell marker in human mammary tumors that correlates very well with tumor grade, metastasis, and cancer stage [209]. Moreover, treating normal human mammary epithelial cells with benzo(a)pyrene caused 3-fold up regulation of only two genes, one of which was *RALDH3* [210]. In HL1-1 human liver cells, 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure induced *RALDH3*, as well as a number of other genes [211]. Although these data seem contrary to the lack of RALDH3 in mammary epithelial tumors, they may reflect a compensatory mechanism to carcinogenic insult, or may reflect differences caused by comparing samples from a small patient population to cells maintained in culture for long durations.

Much less work has been done on regulation of *RALDH3* expression than with *RALDH1* and *2* expression. Phenobarbital induces *RALDH3* 2 to 10-fold in rat liver, depending on the strain [212]. In glioblastoma cells, a fusion protein of IL-13 with a mutated form of Pseudomonas exotoxin induces *RALDH3* via the IL-13 receptor, suggesting a mechanism of regulating atRA biosynthesis [213]. In primary keratinocytes, atRA (1 μM) up regulates *RALDH3* ~10-fold, but does not affect *RALDH1* and *RALDH2* expression [214]. atRA also increases *RALDH3* expression in organotypic human skin cultures and in an epidermal explant, but has no effect in dermal fibroblasts or HeLa cells. The authors proposed that these data suggest a “feed forward” loop, although this really requires much more study to understand, especially because of the high atRA concentration evaluated. Normally, atRA exerts negative feedback on its concentrations.

Dihydrotestosterone induces *RALDH3* mRNA 4-fold in the prostate cell line LNCaP via activating the androgen receptor [215]. An ~8-fold increase in retinal conversion into atRA accompanied *RALDH3* induction, and induction of *CYP26A1* followed the atRA increase, thus indicating androgen regulation of RALDH3 expression and atRA biosynthesis.

16. CYP functions in atRA homeostasis

Several reviews discuss CYP contributions to atRA homeostasis in detail; therefore, this section will refer only to key points, to emphasize participation of CYP in autoregulation of atRA homeostasis [8, 117, 216, 217]. The first observation that atRA degradation contributes essentially to maintaining atRA function involved the demonstration that inhibiting atRA metabolism enhances atRA potency in vivo and in retinoid responsive cells [218-222]. This realization motivated synthesis of a variety of atRA catabolism inhibitors known as retinoic acid metabolism blocking agents, or RAMBA, for treating cancer and dermatological diseases, as an alternative to dosing with atRA or synthetic retinoids, to avoid the toxicity of the later two [223-226].

The importance of atRA degradation to controlling atRA function has been illustrated by knockout of *CYP26A1*, the only atRA-dedicated CYP knocked out to date [227, 228]. *CYP26A1*-null mice die during mid to late gestation with morphological defects that involve the hindbrain, vertebrae, and tailbud. The mutants have spina bifida, tail truncation, and kidney abnormalities, among other deformities, which mimic those produced by treating WT mice with excess atRA. These outcomes contribute to the insight that concentrations of atRA must be tightly controlled spatially and temporarily during development. Whereas developing embryos require atRA, they also require it in measured amounts and/or not at all in some loci, consistent with the well-known teratogenicity of atRA, or of retinol given in sufficiently high doses to defeat control mechanisms and produce atRA pharmacologically.

Interestingly, crossing *CYP26A1*-null mice with *RALDH2*-null mice (heterozygotes) results in 12 of the 17 offspring living until weaning [229]. These data indicate that oxidized derivatives of atRA, such as 4-oxo-RA, 4-OH-RA, and 18-OH-RA, represent catabolites of atRA, and by preventing atRA catabolism, a reduction in atRA generation can be made tolerable. This is the same phenomenon observed in the *RDH1*-null mice, which have diminished *CYP26A1* expression, and illustrates the principle underlying the efficacy of RAMBA [3, 221].

17. ADH1 catalyzes metabolism of high retinol doses

The medium-chain alcohol dehydrogenases, ADH1 and ADH4, convert retinol into retinal *in vitro*, but Han et al. detected no activity of ADH3 with 100 μM retinol and 930 nM enzyme subunit, which confirmed earlier findings of no ADH3 activity with all-*trans*-retinol [58, 230-232]. Using a more sensitive hplc assay, a recombinant ADH3 purified by affinity chromatography had extremely low activity with 30 μM retinol (105 pmol/mg/min)—a concentration that exceeds unbound retinol concentrations in vivo by orders of magnitude (see Figure 3)—and no kinetic values were reported [233]. This rate is unfavorable even compared to *non-purified* RDH. For example, 1 μM recombinant RDH1, using an 800 x g supernatant of CHO cells, generated 2 nmol retinal/mg protein/min. Thus, these data do not establish ability of the soluble ADH3, which does not recognize holo-CRBP1, to access sufficient unbound retinol in vivo (not likely present in the soluble matrix) to contribute to atRA biosynthesis. Moreover, ethanol inhibits atRA production catalyzed by rat liver cytosol with an IC_{50} ~20 μM, but up to 10 mM ethanol does not inhibit microsomal retinol metabolism [51]. This reflects inhibition of the cytosolic ADH by ethanol in vitro. In vivo, however, ethanol dosing does not decrease tissue or serum atRA concentrations; rather, either no changes occur, or increases in atRA occur in select locations [180, 234].

Data have not been published demonstrating ADH catalysis of physiological retinol concentrations in vivo, or metabolism of retinol by ADH expressed in cultured cells, or of impaired ADH expression reducing metabolism of physiological retinol concentrations in vivo or in cultured cells. Conclusions that ADH knockouts reveal function in atRA biogenesis have relied in part on correlations between atRA biosynthesis sites and ADH expression patterns [235, 236]. Correlations have limitations, but these correlations are especially vulnerable, as atRA biosynthesis is widespread among many structures/tissues in the developing embryo and in the adult, and in multiple cell types of both. In fact, ADH1 expression does not correlate well with atRA biosynthesis sites, and ADH4 has restricted expression [237]. ADH3, however, is expressed ubiquitously. Thus, overlap between atRA biosynthesis and ADH3 expression, and ADH4 in some sites, seems assured, which imparts limited insight about causal relationships.

ADH-null mice have not been informative with respect to a function for ADH in atRA generation under physiological conditions. Studies of ADH knockout mice have not reported essential experiments, such as quantifying atRA during normal vitamin A nutriture, documenting phenotypic alterations consistent with reduced atRA, and assaying for gene expression changes indicating lack of atRA signaling or compensation for disrupted atRA function. Thus, genetic, metabolic, and function-related data are not available to support the notion that ADH metabolize *physiological* levels of retinol in vivo.

When stressed by feeding a vitamin A-deficient diet over a longer term, *ADH3* and *ADH4*-null mice do not thrive, whereas the *ADH1*-null mouse remains unaffected [233]. Although this has been interpreted to indicate that ADH3 and ADH4 generate atRA during low vitamin A intake, crucial data are lacking to support this hypothesis. atRA was not quantified in serum or tissues, and the cause(s) of death was/were not reported. The notion

was not discussed that knocking out *ADH3* and *ADH4* causes stress, and vitamin A deprivation itself stresses animals and would compound stress afforded by gene ablation. These observations did not distinguish the possibility that atRA compensates for stress caused by absence of ADH3 and 4, or that additional stress in the absence of atRA causes death by mechanisms unrelated to ADH3 and 4 function, from the proposal that ADH participate in atRA biosynthesis.

More recently, a counterintuitive hypothesis proposes that ADH1 and perhaps ADH2 function to eliminate excess retinol to avoid retinol toxicity [238]. Deliberately generating atRA to avoid retinol toxicity seems analogous to generating prostanoids to avoid polyunsaturated fatty acid toxicity. The issue throughout evolution has been to sequester limited amounts of retinol. Evolutionary pressure has not been exerted in humans, mice or rats to eliminate “excess” retinol; rather the experimental data show clearly that increased dietary retinol leads to increased RE, reaching mM values in liver of rodents fed a chow diet [123]. In addition, CYP occur that recognize retinol as substrate and convert it into more polar products (other than atRA). RA is more toxic than retinol. Retinol/RE may contribute further to retinoid toxicity when organisms are overwhelmed by exposure to vast amounts of vitamin A (a rarity), such that catabolism and generation of RE can no longer relieve the stress and the accumulated retinol and RE begin to disrupt membranes. Unless such rare and extreme conditions, multiple xenobiotic clearing enzymes would catabolize retinoids. The *ADH1*-null mouse has vastly decreased ability to convert high doses of retinol (50 mg/kg) into RA. But this bolus provides ~300-fold more than the recommended daily intake for a mouse, and drives serum atRA ~1600-fold over the steady-state value of ~2 pmol/ml in WT mice. A much lower single retinol dose (3 mg/kg) induces 71% incidence of cleft palate in mice, and a somewhat lower dose (39 mg/kg) induces 76% incidence of neural tube defects [239]. Emphasis has been directed toward a >80% decrease in serum atRA in the *ADH1*-null mouse after the 50 mg/kg dose, but the *ADH1*-null mouse still allows an atRA concentration ~100 to 200-fold greater than normal with this toxic dose. These data, therefore, do not indicate that ADH detoxifies retinol, because prolonged exposure to even modestly increased concentrations of atRA cause teratology [240, 241]. Rather, these data show that high (toxic) vitamin A doses overwhelm homeostatic mechanisms that evolved to concentrate scarce retinol, chaperone its disposition in vivo, and store it as RE and produce toxic amounts of RA.

Liver RE in the double *ADH1/CRBP1* knockout exceed those in the single *CRBP1* knockout, and decline more slowly when the animals are switched from a chow to a vitamin A-deficient diet, indicating that the absence of CRBP1 releases retinol from “supervision” and allows ADH1 access to retinol [242]. Liver RE values in the single *ADH1* knockout exhibited too much variation to compare to WT. One and 5 weeks after switching from a chow to a vitamin A-deficient diet, RE in liver of the single *ADH1*-null differed significantly from WT, but RE values at 9, 13 and 18 weeks did not differ significantly from WT. No simple explanation is apparent for such a phenomenon, and the large SE and few mice/group (n = 3) contribute to confounding interpretation. It is not clear which of these points may be valid, but if the last three points are, then the data do not support the contention that ADH1 serves a physiological function to deplete RE. Also, recall that the amount of dietary vitamin A and the STRA6/LRAT/CRBP1 mediated uptake and esterification of retinol determines the liver RE concentration. In addition, ethanol, a competitive inhibitor of ADH1, increases liver atRA within 1 hr after dosing [180]. In summary, retinol metabolism catalyzed by ADH likely *contributes* to retinoid toxicity by generating high concentrations of atRA during exposure to vitamin A doses that saturate CRBP1 and defeat the mechanism of innocuous retinoid storage as RE.

18. Summary

A plethora of biochemical and genetic data consistently identify precise functions for the retinol dehydrogenases, RDH1, RDH10, and perhaps DHRS9 as physiological catalysts of the first step in atRA biosynthesis. Much data also conclusively identify RALDH1, RALDH2, and RALDH3 as participating in the second and irreversible step. Each of these enzymes has been conclusively related to a biological process mediated by atRA. Most of these enzymes interact with retinoid binding-proteins and, in several cases, influence the expression of one another. The cumulative data support the model of Figure 1 that proposes interactions among these enzymes and retinoid binding-proteins, with feedback regulation and/or control by atRA via (tissue specific) modulation of *RALDH*, *CYP*, *LRAT*, *STRA6*, *and CRBP1*. In addition to transcriptional regulation, the ratio apo-CRBP1/holo-CRBP1 likely influences flux of retinol into and out of RE. Finally, preliminary data suggest post-translation regulation of at least RALDH1. Understanding where and when atRA biosynthesis occurs increasingly depends on placing the topic into the larger context of the associated enzymes and binding proteins, rather than focusing on a single contributor to this intricate process.

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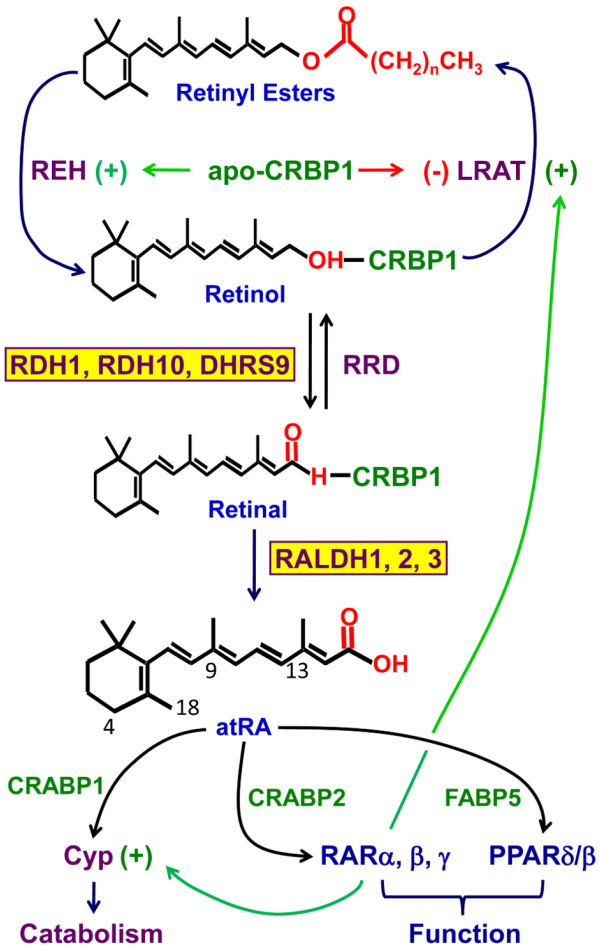


Figure 1. An integrated model of atRA homeostasis

This model accounts for the fact that the very high-affinity retinol binding-protein, holo-CRBP1, represents the major physiological form of intracellular retinol, and apo-CRBP1 interacts with enzymes to signal the intracellular concentration of retinol. The model presents the major enzymes that catalyze the rate-limiting step, retinol dehydrogenation, and the irreversible step, retinal dehydrogenation. These enzymes all have been validated biochemically, physiologically, and genetically, as contributing to atRA biosynthesis under physiological conditions. The model also postulates functions for the atRA binding proteins, CRABP1, CRABP2, and FABP5 in delivering atRA to distinct nuclear receptors or for catabolism. Finally, two of the autoregulatory nodes activated by atRA are shown, induction of CYP and LRAT to lower atRA and retinol concentrations, respectively.

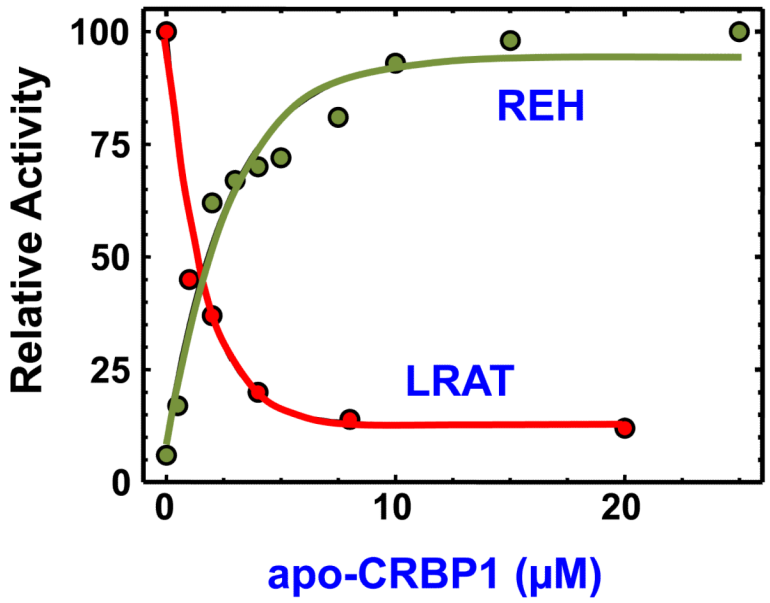
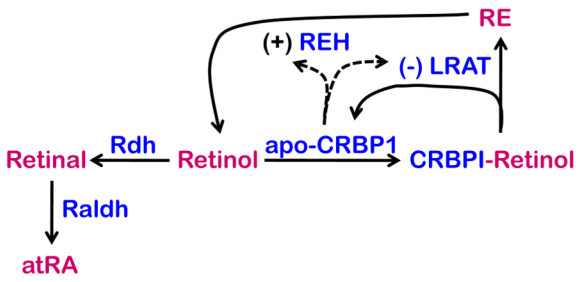


Figure 2. Concentration effects of apo-CRBP1 in the stimulation of retinyl ester hydrolase (REH) and inhibition of RE biosynthesis

Apo-CRBP1 (apoCRBP) activates hydrolysis of endogenous RE in microsomes by a cholate-independent REH [32]. Half-maximum stimulation occurs ~2.6 μM. apo-CRBP1 also serves as a potent competitive inhibitor of LRAT, with 50% inhibition occurring ~2 μM [33]. Thus, the ratio apo-CRBP1/holo-CRBP1 would serve as a signal of intracellular retinol status to influence the relative rates of RE biosynthesis vs. RE hydrolysis.

A



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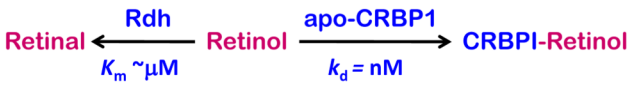

C

ROH_{free} (μM)	holo-CRBP1 (μM)	k_d (nM)	ROH_{total} (μM)	$CRBP1_{total}$ (μM)
0.008	4.992	3.2	5	7
0.0004	4.999	0.1	5	7
0.025	1.925	3.2	2	2
0.015	1.985	0.1	2	2

Figure 3. An alternative model of generating atRA

A) This model indicates a possible alternative to that shown in Figure 1, without direct involvement of holo-CRBP1 delivering retinol to RDH and RALDH, which requires diffusion of retinol from its site of release by holo-CRBP1. B) This would require competition between RDH and apo-CRBP1 for retinol (and between RALDH and apo-CRBP1 for retinal). The very high affinity of apo-CRBP1 (nM) for retinol would severely restrict RDH access to retinoids, especially with the driving force of RE formation mediating intracellular retinol uptake (see A). C) Shown are the effects of CRBP1 affinity (k_d values) on concentrations of unbound (or free) retinol). With the measured k_d value of ~3 nM (considered a high estimate because of technology limitations), the available free retinol concentration would vary between 8 and 25 nM, depending on the ratio of total CRBP1 to total retinol. With a k_d value of ~0.1 nM, considered as likely more accurate, free retinol concentrations would vary between 0.4 and 15 nM.

Table 1

Rdh that contribute physiologically to atRA biosynthesis. Rows depict orthologs Columns depict homologs and/or paralogs

Mouse	Rat	Human
Rdh1	Rdh7 and Rdh2 (originally RodhI and RodhII)	Rdh16 (originally Rodh4, RDH-E)
Rdh10	Rdh10	Rdh10
Dhrs9	Dhrs9 (originally eRolDH2)	Dhrs9 (originally retSdr8, RDHL, Rdh-TBE, RoDH-E_2, 3α-HSD

Table 2
Representative retinoid concentrations in mice (nmol/g or ml)

Retinoid	Liver	Serum	Exptrahepatic tissues
RE	500[a]	0.2	0.2-1.8
Retinol	10	0.8	0.08-0.8
Retinal	0.16	0.03	0.06-0.19
atRA	0.04	0.003	0.002-0.02

[a]The dietary content of vitamin A affects RE much more so than the other values, but all values may to an extent reflect diet and strain [34, 44].