Title

Vitamins | Vitamin A (Retinoids) ☆

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21374 Vitamin A (Retinoids)[☆][☆]

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Glossary

K_d **(equilibrium dissociation constant)**   A measure of the affinity of a protein for its ligand. Lower numbers indicate higher affinity.
Retinoid binding-protein   Proteins that bind specific retinoids with high affinity, several with K_d values ≤10 nM.
Short-chain dehydrogenase/reductase (SDR)   A gene family consisting of ~50 mammalian members in the range of 25–35 kDa that uses pyridine nucleotide cofactors to dehydrogenate or reduce steroids, retinoids, prostanoids, and intermediates in lipid metabolism.

The History of Vitamin A

A history of the discovery of vitamin A has been related in detail by **Wolf (1996)**. Readers are referred to this article to learn the progression of insight into a factor understood by ancient civilizations to treat night blindness (nyctalopia) and xeroxerophthalmiathalmia (degeneration of the cornea), and the ultimate discovery of the chemical substance responsible for the physiological functions of retinoids. The WoWolfld article progressers to discoveries in the modern age, including the retinoid binding-proteins and retinoid receptors. It is an interesting read.

Vitamin A/Retinoid Functions

The term retinoid refers to naturally occurring and synthetic compounds with vitamin A activity: the term vitamin A denotes the compound all-*trans*-retinol (retinol). Vertebrates require vitamin A for vision, fertility, embryogenesis, regulation of energy balance, and growth (**Wiseman *et al.*, 2017**). Retinol itself does not fulfill the physiological functions of vitamin A; rather retinol serves as precursor for biosynthesis of metabolites directly responsible for producing "vitamin A activity." These metabolites include 11-*cis*-retinal, the cofactor that covalently binds opsin to form the visual pigment rhodopsin (**Saari, 2016**); and all-*trans*-retinoic acid (RA), the humoral effector of the non-visual, systemic functions attributed to vitamin A (Napoli, 2012; Kedishvili, 2016a,b); and 9-*cis*-RA (9cRA), a regulator of glucose-stimulated insulin secretion (**Cione *et al.*, 2016**). These systemic functions include: controlling differentiation programs of stem cells, apoptosis, and/or proliferation in skin and bone and in the nervous, hemopoetic, epithelia and reproductive systems (Beckenbach *et al.*, 2015; Green *et al.*, 2016; Griswold, 2016; Teletin *et al.*, 2017); acting as a tumor suppressor (**Uray *et al.*, 2016**); regulating energy balance, macronutrient metabolism and adiposity (Frey and Vogel, 2011; Noy, 2013; **Fig. 1**). Non-visual cycle effects of retinoids also include memory formation (**Stoney and McCaffery, 2016**) and immune function (**Brown and Noelle, 2015**). RA and 9cRA activate three nuclear receptors, RARα, β and γ, and thereby control expression of hundreds of genes (**Iskakova *et al.*, 2015**). RA also ligands with PPARδ/β to induce genes that differ from those induced by RAR (**Noy, 2016a**). Both RAR and PPAR heterodimerize with RXR, and as such are type II nuclear receptors. The RA-binding protein Crabp2 delivers RA to RAR, whereas the "fatty acid" binding protein FABP5 delivers RA to PPARδ/β, thereby distinguishing RA pro-differentiation effects from its proliferation effects.

RA also functions through non-genomic mechanisms. Cytosolic holo-Crabp1 (cellular RA-binding protein complexed with RA) negatively regulates proliferation of the hippocampal neural stem cell population, through the RAF/MEK/ERK signaling path (**Wei, 2016**). This has the effect of enhancing RA-induced differentiation that occurs via RAR. This non-canonical action of holo-Crabp1 also functions in differentiated cardiomyocytes to dampen excessive CaMKII action, preventing cardiac damage. In contrast, holo-Crabp2 chaperones RA to RAR in the nucleus and cycles back to cytosol as apo-Crabp2 (Vreeland *et al.*, 2014; Zhang *et al.*, 2016). In cytosol, apo-Crabp2 binds to the mRNA binding-protein HuR. Hur associates with mRNA 3′-untranslated regions, which stabilizes mRNA. Apo-Crabp2 enhances affinity of HuR for mRNA, further increasing HuR action.
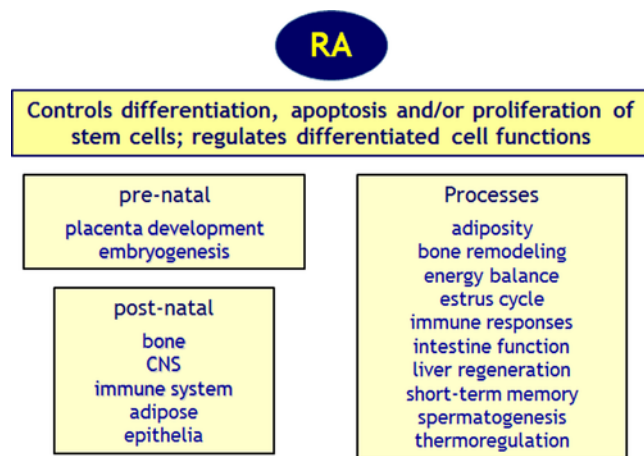

Fig. 1   Systemic functions of all-*trans*-retinoic acid (RA) illustrating pre- and post-natal sites of action and processes affected.

Retinoid Generation From Carotenoids and Retinoid Storage

Carotenoids with at least one β-ionone ring, but especially β-carotene with two β-ionone rings, provide the major retinoid precursors in most diets. Oxidative central cleavage by a carotene 15,15′-dioxygenase (Bco1) produces all-*trans*-retinal (dela Seña *et al*., 2014; Harrison, 2019). Retinal reductases (Dhrs3, Dhrs4, Rdh11), members of the short-chain dehydrogenase/reductase (SDR) gene family, reduce all-*trans*-retinal into retinol. Retinol undergoes esterification with fatty acids, predominately palmitate and oleate, into all-*trans*-retinyl esters (RE), catalyzed mainly by lecithin: retinol acyltransferase (Lrat) (Harrison, 2005; Sears and Palczewski, 2016). In some situations, acyl-CoA: diacylglycerol acyltransferase 1 (Dgat1), formerly known as acyl-CoA: retinol acyltransferase (Arat), also catalyzes RE biosynthesis (Shih *et al*., 2009; Kaylor *et al*., 2015). Chylomicrons transport RE formed in the small intestine into circulation and deliver some to adipose, whereas chylomicron remnants deliver most to liver. In liver, most RE are stored in stellate cells (**Saeed *et al*., 2017**). Most tissues, however, harbor some RE.

The serum Rbp, encoded by Rbp4, a member of the lipocalin gene family, delivers retinol from liver to the retina and other extra-hepatic tissues (Noy, 2016b; Harrison, 2019). Retinol egress from liver requires Rbp: the complex RBP-retinol represents the major form of vitamin A in serum. Rbp circulates as a complex with a tetramer of transthyretin, which protects the ~20 kDa Rbp from degradation and kidney excretion. The Rbp4-null mouse seems phenotypically normal when fed a diet that contains copious amounts of vitamin A (~30 IU/g), except for impaired vision after weaning. Feeding a diet with lower, but adequate amounts of vitamin A, prevents reproduction in the Rbp4-null mouse. Although the eye relies on Rbp for efficient retinol delivery, retinol obtained from postprandial lipoprotein delivery can substitute, if the animals are fed diets containing copious amounts of vitamin A. Interestingly, RA serum levels increase in the Rbp4-null mouse even during copious vitamin A diets, indicating that serum delivery of RA to tissues helps compensate for impaired retinol delivery.

The plasma membrane receptor Stra6 (stimulated by RA6) recognizes Rbp4 and transports its retinol into cells (**Kawaguchi *et al*., 2015**), where the retinol is esterified into RE by Lrat. Eye expresses Stra6 most intensely, followed by CNS and testis (**Kelly and von Lintig, 2015**). Many vitamin A-target tissues do not express Stra6 or express it in low levels, suggesting alternative mechanisms for sequestering retinol from blood. Some tissues low or absent in Stra6 express the alternative Rbp receptor Rbpr2 (**Alapatt *et al*., 2013**). These tissues include liver and intestine. In vitamin A target tissues, retinol undergoes conversion into RA in a two-step process; the first, generation of retinal, is rate-limiting. **Fig. 2** summarizes overall biogeneration and function of RA.

The Visual Cycle

To support vision, all-*trans*-RE in the retinal pigment epithelium undergo concerted hydrolysis-isomerization into 11-*cis*-retinol catalzed by Rpe65. Rpe65 belongs to the same gene family as the carotenoid-metabolizing enzyme Bco1, consistent with a family devoted to metabolism of hydrophobic substances. Dehydrogenation of 11-*cis*-retinol into 11-*cis*-retinal by the SDRs Rdh5 and 10 provide cofactor to create rhodopsin (**Sahu and Maeda, 2016**). Mutations in Rdh5 associate with the rare autosomal recessive disease fundus albipunctatus, i.e. night blindness from delayed photopigment regeneration (**Sergouniotis *et al*., 2011**). Lack of total blindness in the case of the Rdh5 mutations indicate a contribution of the SDR Rdh10 to 11-*cis*-retinal biosynthesis. 11-*cis*-Retinal transverses the interphotoreceptor matrix by an unknown mechanism and enters the photoreceptors (rods and cones), where it forms a Schiff's base with a lysine residue in the protein opsin to form rhodopsin (**Fig. 3**). When light strikes the neural retina, 11-*cis*-retinal in rhodopsin undergoes *cis* to *trans*-isomerization, causing a conformation change in rhodopsin that initiates a nerve impulse through activation of G-
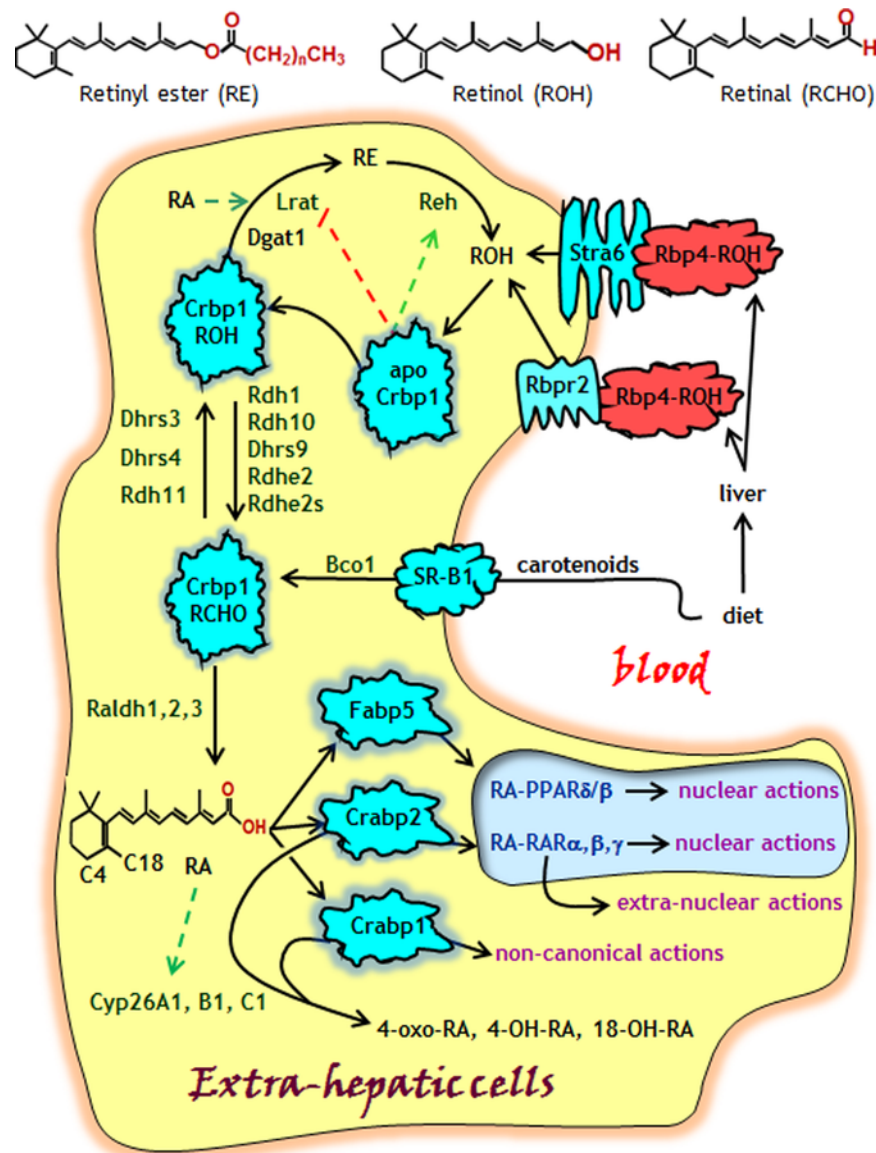
Fig. 2 RA generation and mechanisms of action. Background: Diet delivers retinyl esters via chylomicrons to adipose and liver, eventually to be stored in stellate cells. Diet also delivers carotenoids to multiple tissues to undergo central cleavage into retinal. Liver stellate cells store most RE, but adipose also store a portion of RE, and most other tissues have detected RE. Rbp4 (serum Rbp) in the liver acquires retinol co-translationally and enters circulation to associate with transthyretin. Start of illustration: Rbp4 delivers retinol to extra-hepatic cells by binding with Stra6. Not all retinoid target tissues express Stra6, eye having the most intense expression. Some tissues that do not express Stra6 express a different Rbp4 receptor, Rbpr2. Crbp1 causes retinol release from Stra6 to proceed in the direction of uptake, by sequestering retinol and directing it to Lrat for conversion into retinyl esters (RE). Lrat catalyzes RE bioformation in virtually all tissues. In a few locations, however, such as skin, diacylglycerol acyltransferase 1 (Dgat1) also contributes to RE formation. Crbp1 also channels retinol to retinol dehydrogenases (Rdh), which convert retinol into retinal. Crbp1 then allows access of retinal to retinal dehydrogenases (Raldh, encoded by Aldh1a1,2,3) for irreversible conversion into retinoic acid (RA). Carotenoids provide most vitamin A. Carotenoids enter cells via scavenger receptor class B1 (SR-B1). Pro-vitamin A carotenoids are cleaved centrally by β-carotene cleavage enzyme 1 (Bco1) into one or two molecules of retinal, depending on the nature of their β-ionone rings. Retinal reductases convert retinal into retinol, and contribute to maintaining RA homeostasis and preventing RA toxicity. Three binding proteins sequester RA, Crabp1, Crabp2 and Fabp5. All belong to the fatty-acid binding-protein gene family. These binding-proteins have distinct functions in delivering RA to nuclear receptors (PPAR, RAR), or in mediating non-genomic actions (Crabp), and/or in delivering RA to cytochrome P450s (Cyp) for catabolism (Crabp). RA limits its own concentrations by inducing Cyp and Lrat (green arrows). The ratio apo-Crbp1/holo-Crbp1 reflects cells' retinol status and regulates retinol flux via apo-Crbp1 inhibiting Lrat and inducing retinyl ester hydrolases (Reh). Liver has the same apparatus of extra-heptatic cells, but doe not rely on deliver of retinol by Rbp receptors.
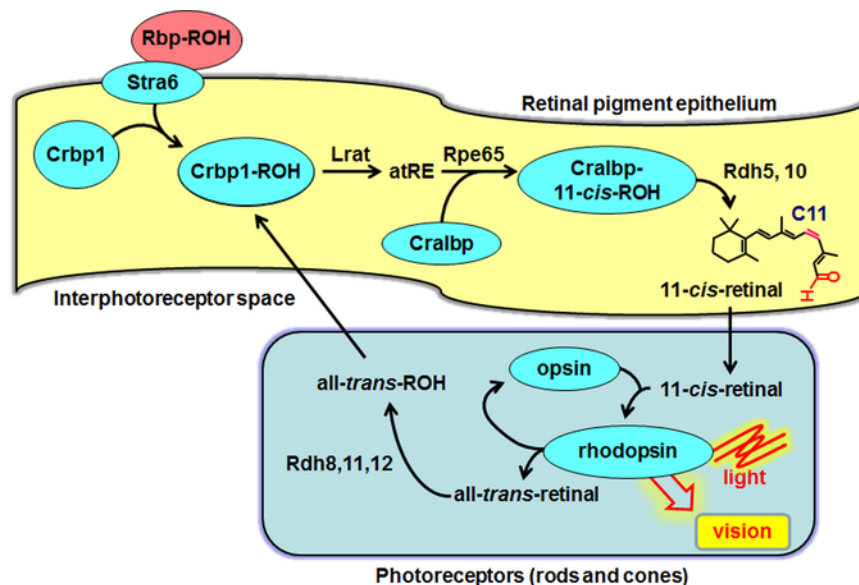
Fig. 3 The visual cycle. The retina consumes most vitamin A used daily. Serum Rbp (encoded by *Rbp4*) delivers retinol to the retinal pigment epithelium (RPE) via Stra6. Crbp1 sequesters retinol and channels it to Lrat to produce all-*trans*-retinyl esters (atRE). A concerted reaction catalyzed by Rpe65 converts atRE into 11-*cis*-retinol, which binds with cellular retinal binding-protein (Cralbp). Cralbp channels 11-*cis*-retinol to Rdh, which biosynthesize 11-*cis*-retinal. 11-*cis*-Retinal relocalizes to the photoreceptors, in which it forms a Schiff's base with the protein opsin to form rhodopsin. Light striking rhodopsin isomerizes 11-*cis*-retinal to all-*trans*-retinal. The "straightening" of the isoprenoid structure changes the conformation of rhodopsin, resulting in release of all-*trans*-retinal and generation of a nerve impulse, resulting in vision. Retinal reductases reduce all-*trans*-retinal to all-*trans*-retinol, which relocates to the RPE.

proteins. This conformation change also releases the newly re-formed all-*trans*-retinal. An ATP-dependent transporter, ABCR, facilitates release of all-*trans*-retinal from rhodopsin. The SDRs Rdh8, 11 and 12 reduce all-*trans*-retinal into retinol in the photoreceptors. Retinol migrates back to the retinal pigment epithelium, where Lrat re-esterifies it into RE (Saari, 2016; Daruwalla *et al*., 2018).

Activation of Retinol into Retinoic Acid (RA)

Activation of retinol into RA proceeds through the same intermediate used in the visual cycle, all-*trans*-retinal, but relies on a metabolically distinct route. Retinol, either from blood or from hydrolysis of RE by RE hydrolases (Reh) (**Grumet *et al*., 2016**), undergoes reversible dehydrogenation into all-*trans*-retinal, catalyzed by retinol dehydrogenases, Rdh (Rdh1, Rdh10, Dhrs9, Rdhe2, Rdhe2s), members of the SDR gene family (Kedishvili, 2016a,b; Napoli, 2016). In contrast to the comparatively high concentrations of retinal required for the visual cycle, all-*trans*-retinal concentrations during RA biosynthesis are kept low (50–200 nmol/g) by reduction (reduction by microsomal and peroxisomal reductases, Dhrs3, Dhrs4, Rdh11), and by irreversible dehydrogenation into RA, catalyzed by retinal dehydrogenases (Raldh1, 2, 3), members of the aldehyde dehydrogenase (Aldh: Aldh1a1, 1a2, 1a3) gene family. RA isomers occur in vivo, such as 13-*cis*-RA and 9,13-di-*cis*-RA, but their significance and source(s) remain unclear. Recently, the issue of physiological occurrence of 9cRA as a *bona fide* hormone has been resolved with its analytically rigorous identification in the pancreas by LC/MS/MS, even though it has not been detected (<0.05 pmol/g) in a variety of other retinoid target tissues (**Kane *et al*., 2010**).

Knockouts of *Rdh1* and *Rdh10* have phenotypes related to dysfunctions of retinoid-regulated genes and retinoid action (Zhang *et al*., 2007; Yang *et al*., 2018; Krois *et al*., 2019). The *Rdh1*-null mouse gains up to 37% more weight than wild-type as adipose, due to dysfunctional brown adipose, even when fed a low-fat diet. In contrast to the Rdh1-knockout, the homozygotic *Rdh10*-null mouse dies during embryogenesis, because of impaired brain development. This effect of ablating *Rdh10* occurs during a very limited time of embryogenesis, consistent with contributions of additional Rdh. The heterozygous *Rdh10* knockout has numerous metabolic dysfunctions in liver, bone, muscle and adipose, and gains excess weight relative to wild-type only when fed a high-fat diet. *Rdh1* and *Rdh10* have different subcellular expression patterns and do not exhibit the same phenotypes when ablated. This suggests each generates RA for specific vitamin A functions.

Raldh catalyze the irreversible conversion of all-*trans*-retinal into RA in the presence of Crbp1, and also accesses retinal generated in situ from Crbp1-retinol by Rdh, or in cells presented with retinol and transfected with Rdh and Raldh (**Napoli, 2012**). Raldh1 and Raldh2 have differing, but overlapping, expression patterns, respond differently to changes in retinol status, and have different but overlapping subcellular expression patterns. This suggests a purpose for multiple Raldh, i.e.*,* precise temporal and locational control over retinol use and RA generation.

The *Raldh1*-null mouse remains fertile and healthy, but has decreased ability to produce RA in the liver, although adipose RA generation is normal (**Yang *et al*., 2017**). In contrast to the *Rdh1*- and *Rdh10*-null mice, the *Raldh*-null mouse resists adiposity when fed a high-fat diet. This phenotype contrasts with *Rdh*-null phenotypes, and should be considered in context of: (1) no decrease in adipose RA biosynthesis by ablating *Raldh1*; (2) the multiple substrates and functions of Raldh1. These include as a cancer stem cell marker, including dopamine and its metabolites, oxazaphosphoranes and aldehyde lipid peroxidation products, serving as an androgen binding-protein, the eta-crystalline of the cornea and lens of mammalian eyes. These data indicate that Raldh1 does not promote adiposity via retinoid signaling. (Cai *et al*., 2014; Tomita *et al*., 2016; Yang *et al*., 2017). The *Raldh2*-null mouse dies *in utero* at midgestation, demonstrating its unique contribution to RA biosynthesis at a specific time during embryogenesis. The situation may differ in the adult, as testes express *Raldh 1*, *2*, *3*, and *4* (**Kent *et al*., 2016**), but Raldh1 prevails outside of the testis. The *Raldh3*-null mouse dies during suckling from an obstruction in the nose. Apparently, Raldh can compensate for each other after critical developmental milestones.

These multiple activating enzymes offer complex opportunities for physiological regulation, owing to compartmentalization, distinct enzymes catalyzing each direction of reversible reactions (e.g., dehydrogenation/reduction of retinol/retinal; esterification/hydrolysis of retinol/RE), cell-distinct expression patterns, and multiple homologs for each of most reactions.

Retinoid Binding-Proteins and Retinoid Metabolism

Processing of dietary retinoids and retinoid precursors, and biogeneration of active retinoids, relies on cellular chaperones for efficient and specific retinoid use, as demonstrated by studies in vitro and the phenotypes of gene knockouts and/or naturally-occurring mutations.

Cellular retinoid binding-proteins channel retinoids through the series of reactions that constitute the visual cycle (**Fig. 3**). Crbp1 (cellular retinol binding-protein type 1) in the retinal pigment epithelium garners retinol from Stra6 and channels it to Lrat for esterification. The presence of Crbp1 guarantees one-directional intake of retinol through Stra6. Esterification by Lrat discharges Crbp1, thus continuing one-directional uptake physiologically. Rpe65 catalyzes a concerted reaction that hyrolyzes RA and isomers the C11 double bond (**Saari, 2016**). The Rpe65 product, 11-*cis*-retinol, undergoes sequestration by cellular retinal binding-protein (Cralbp), a member of the gene family that includes the α-tocopherol transfer protein (TTP). Cralbp channels 11-*cis*-retinol to Rdh5 and 10 for conversion into 11-*cis*-retinal, and thereby drives forward the *trans* to *cis* isomerization. Mutations in human Cralbp cause night blindness and photoreceptor degeneration.

Outside of the visual cycle, multiple cellular retinoid binding-proteins chaperone retinol and RA (**Napoli, 2016**). These include: Crbp1, Crbp2, Crbp3, Crabp1, and Crabp2, which are ~15 kDa globular proteins of the intracellular lipid binding-protein (iLbp) gene family. Vertebrates express all five, with well-conserved amino acid sequences among orthologs. All are high-affinity (except Crbp3) and specific for their ligands. Crbp1 binds retinol with high affinity ($k_d \leq 1$ nM), and closely related compounds such as 3,4-didehydro-retinol and, with lower affinity, all-*trans*-retinal. Crbp's discriminate against RA and its isomers. Crabp1 and 2 bind RA, and metabolites such as 4-OH-RA, 4-oxo-RA, and 18-OH-RA, but discriminate against *cis*-RAs and retinol.

Cellular retinol/retinal binding-proteins enclose retinol and all-*trans*-retinal sheltering inside the hydroxyl/aldehyde function, crucial to metabolic activation. Crbp1 and 2 confer selective advantage to vertebrates by enhancing efficiency of storing and transporting vitamin A and limiting its catabolism. Vitamin A absorption and biosynthesis in the intestine relies on Crbp2. *Crbp2*-null mice pups die within 24 h after birth, when delivered by dams fed a diet marginal in vitamin A content (a usual situation during evolution) (**E *et al*., 2002**). Crbp2 contributes ~1% of the soluble protein to the intestinal enterocyte – an indication of a mass-action function to sequester newly synthesized (from carotenoids) all-*trans*-retinal or newly absorbed dietary retinol. All-*trans*-retinal bound with Crbp2 undergoes reduction readily, but neither it nor bound retinol undergoes efficient dehydrogenation (**Kakkad and Ong, 1988**). This limits production of RA by the intestine from the bolus of all-*trans*-retinal produced during carotenoid uptake and cleavage. In the intestine, Lrat recognizes retinol bound with Crbp2 to produce RE for incorporation into chylomicrons. Thus, Crbp2 likely aids carotenoid and retinol uptake by mass action, and chaperones the products of carotenoid metabolism down the pathway to RE to enhance efficiency of retinoid recovery from the diet.

Extra-intestinal vitamin A uptake and storage relies on Crbp1 (**Noy, 2016b**). Crbp1-null mice seem morphologically normal, but eliminate RE 6-fold faster than wild-type mice, and may sequester/esterify retinol less efficiently (**Matt *et al*., 2005**). Crbp1-null mice also have impaired glucose tolerance (**Kane *et al*., 2011**). Clearly, efficient use of retinol in vivo depends on this chaperone. Like Crbp2, Lrat accesses retinol bound with Crbp1 to produce RE. Ultimately, liver stellate cells accumulate most RE. Crbp1 seems necessary for retinoid transfer from hepatocytes to stellate cells, because the *Crbp1*-null mouse does not accumulate RE in stellate cells.

In contrast to Crbp2, Crbp1 allows dehydrogenation of retinol into retinal. The ability of Lrat and Rdh to access retinol from Crbp1 (direct transfer, rather than diffusion through the aqueous medium) addresses the issue of how retinol would undergo efficient metabolism in the face of limited diffusion from the binding-protein. The Crbp1-retinol complex displays Michaelis–Menton relationships with RE formation by Lrat and retinol dehydrogenation by Rdh. Specific crosslinking of holo-Crbp1 with both Rdh and Lrat confirms close proximity of Crbp1 and these two enzymes. Additionally, a single mutation in an exterior residue of Crbp1 (L35A) reduces the Vm of retinol dehydrogenation, but does not alter the K_m, or the k_d of retinol binding, consistent with conservation of exterior residues that aid transfer of retinol from Crbp1 to enzymes. RE and RA biosynthesis in vivo can occur in the absence of Crbp1, as indicated by the lack of obvious morphological pathology in the *Crbp1*-null mouse, and their ability to sequester RE, albeit inefficiently. This was predicted by the experiments in vitro, which showed that neither Rdh nor Lrat require presentation of retinol by Crbp1. Not surprisingly, the enzymes' active sites recognize their substrates in the absence of Crbp1. Thus, Crbp1 operates as a chaperone, which restricts retinol metabolism to select enzymes, and seems required for efficient retinol use.

A Crbp3 has been detected in limited tissues, but has not been detected in some retinoid target tissues, such as liver, kidney, brain, etc. Crbp3 seems to bind about equally well with retinol, 9-*cis*-retinol and 13-*cis*-retinol, but with much lower affinity (~109 nM) than either Crbp1 or Crbp2 (**Kono and Arai, 2015**). Ablation of Crbp3 demonstrated that it contributes to optimal incorporation of RE into milk

and enhancing food intake, which contributes to adiposity (**Piantedosi *et al*., 2005**). Humans express yet another Crbp, originally referred to as Crbp3, but distinct from mouse Crbp3, and therefore Crbp4 (**Nishiwaki *et al*., 1990**). Crbp4 mRNA is much more abundant in human liver and intestine than Crbp1 mRNA, but the mouse does not encode a complete Crbp4 gene. Crbp4 binds retinol with a k_d value of ~60 nM, and does not bind *cis*-isomers. The precise functions of Crbp4 have not been clarified.

To summarize, Crbp provide for one-way retinol transfer into cells, and channel retinol to Lrat and retinol/retinal to SDR. Crabp provide for RA catabolism by channeling RA to Cyp26, mediate non-genomic actions of RA, and deliver RA to RAR (Crabp2).

Hormesis and Metabolism of High-Dose Retinoids

RA binding to multiple binding-proteins and nuclear receptors helps explain the multiplicity of its effects. Hormesis also contributes to contrasting retinoid effects. Retinol and RA exhibit the phenomenon of hormesis, i.e., the nature of their effects depends on amounts dosed and the tissue concentrations achieved (Hayes, 2007; Bhakta-Guha and Efferth, 2015; Calabrese *et al*., 2015). Inverted U or J-shaped dose-response curves denote hormesis: as concentrations of a compound exhibiting hormesis increase, beneficial effects increase until reaching a "goldilocks" zone (**Fig. 4**). As concentrations increase further, beneficial effects subside and atypical or toxic effects develop. Therefore, use of copious retinol/RA can confound physiology with pharmacology and/or toxicology.

A related issue involves medium-change alcohol dehydrogenases (Adh), which metabolize high concentrations of retinol in vitro. Adh, however, cannot access Crbp-bound retinol, consistent with evolution of Crbp to protect the scarce and valuable vitamin A from clearance as a xenobiotic. Essential experiments have not been reported for the AdhI-null deermouse, a natural mutant, and mice made null in AdhI, AdhIII or AdhIV or a combined knockout of six mouse Adh. These knockouts have not been shown to exhibit: (1) phenotypes resulting from inadequate vitamin A; (2) compensatory responses in retinoid metabolic enzymes; (3) dysregulated retinoid-responsive genes. Thus, published reports of Adh knockouts do not support physiological involvement of Adh in retinol metabolism (**Napoli, 2016**). In contrast, studies of Adh knockouts suggest a mechanism for retinol toxicity. A single retinol dose of 3 mg/kg dose induces 71% incidence of cleft palate; a 39 mg/kg dose induces 76% incidence of neural tube defects (**Biesalski, 1989**). Dosing 50 mg/kg or ~300-fold > than the recommended daily intake to mice produces a serum RA concentration ~1600-fold > normal. In the AdhI-null mouse, this is reduced to ~200-fold > than normal (still a very toxic situation). These data show that huge amounts of retinol can overwhelm physiological chaperones. These data indicate a mechanism for retinol toxicity, as it takes only modest increases in RA to cause retinoid toxicity.

Other Retinoids

Discrete loci, such as skin and the chick limb bud, synthesize 3,4-didehydro-atRA. 3,4-Didehydro-RA binds RAR with affinity similar to that of RA. The purpose of creating a signaling molecule that functions similar to RA in specialized loci that also biosynthesize RA has not been clarified, but subtle differences in ligand structure can have significant differences on the conformation of nuclear receptors. These differences can lead to recruitment of different cofactors and/or binding to different response elements. Development of synthetic retinoids that act as RAR agonists, antagonists, or inverse agonists illustrate this principle (**le Maire *et al*., 2012**). It is reasonable to infer that RA and 3,4-didehydro-atRA might regulate different genes, even though both bind RAR.
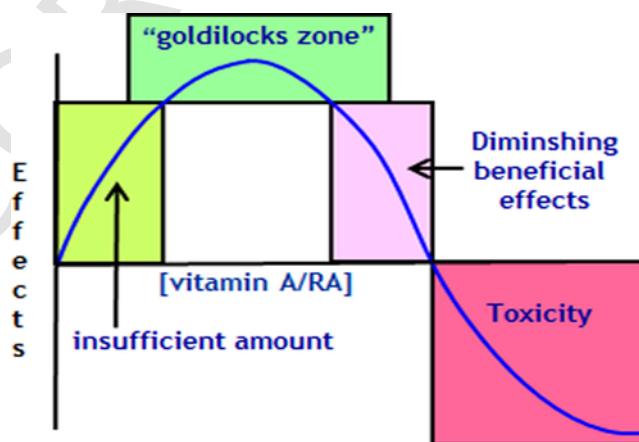

Fig. 4   Vitamin A and RA exhibit hormesis. The amount of vitamin A/RA ingested and in tissues determines effects through the phenomenon of hormesis. Inadequate vitamin A results in growth retardation, blindness due to epithelial degeneration (xerophtalmia) and many other systems' failures. A relatively compressed range of concentrations provides optimum beneficial effects. As concentrations increase beyond this "goldilocks" zone, beneficial effects recede, ultimately reaching concentrations that exhibit toxicity and/or teratogenicity.

Although 9cRA was reported as a physiological ligand of RXR, it is undetectable in vivo in the vitamin A-target tissues assayed, except for pancreas. It, therefore, is unlikely to serve as a universal ligand for RXR, but it does modulate glucose-stimulated insulin secretion by pancreatic β-cells.

Regulation of RA Homeostasis

RA regulates its own homeostasis by inducing *Cyp26A1*, *B1* and *C1* transcription (Ross and Zolfaghari, 2011; Isoherranen and Zhong, 2019). The β-ionone ring of RA projects from Crabp1 and 2 allowing access of Cyp to the C4 and C18 positions, which are the sites of the first reactions of RA catabolism (**Fiorella *et al.*, 1993**). Presenting RA to microsomes (source of Cyp) bound with Crabp1 enhances kinetic efficiency (k_{cat}/K_m) of catabolism 7-fold by fostering direct transfer from the binding protein to catabolic enzymes. Crabp2 also delivers RA to catabolism via direct transfer to Cyp26 (**Nelson *et al.*, 2016**). Delivering Crabp-sequestered RA for efficient catabolism seems an obvious mechanism to discharge the ligand without releasing it back into the cell, and thereby controlling intracellular RA concentrations. Conversely, inhibitors of RA metabolism enhance RA potency. This was first demonstrated with the Cyp inhibitor ketoconazole, which caused a left-ward shift in the dose-response curve of RA in F9 cells, i.e., increased RA potency (**Williams and Napoli, 1985**). This observation engendered syntheses of RAMBAs (RA metabolism blocking reagents) as potential therapeutic agents, to achieve a modest increase in tissue RA that would increase therapeutic efficacy, while avoiding toxicity (**Nelson *et al.*, 2013**).

Other primary regulators of retinoid homeostasis include apo-Crbp1 and insulin. apo-Crbp1 inhibits Lrat and stimulates RE hydrolysis. Thus, the direction of flux into or out of RE reflects the ratio apo-Crbp1/holo-Crbp1, which reflects the cell's retinol status. Moreover, apo-Crbp1 and Lrat activity establish the one-way uptake of retinol into cells from serum (**Noy, 2016b**). Insulin has been identified as a restrictor of RA biosynthesis by expelling the transcription factor FoxO1 from the nucleus (**Obrochta *et al.*, 2015**). Because insulin and RA have contrasting actions (RA induces gluconeogenesis and lipolysis), decreasing RA during the fed state would contribute to augmenting insulin action.

Several xenobiotics, including ethanol and polychlorinated aromatic hydrocarbons reduce RE stores, likely through enhancing RA catabolism by inducing CYP (Napoli, 2011; Clugston and Blaner, 2012; Mahiout *et al.*, 2017).

Retinoids in the Clinic

Numerous studies have correlated vitamin A insufficiency in laboratory animals with increased incidence of spontaneous and carcinogen-induced cancer. Chemopreventive trials in humans show promise for retinoids in actinic keratoses, oral premalignant lesions, laryneal leukoplakia, and cervical dysplasia. The FDA has approved retinoids for acute promyelocytic leukemia and in non-life-threatening diseases such as cystic acne and psoriasis. Retinoids also provide the active ingredients in agents to treat sun/age damaged skin, through regulating transcription, differentiation, and cell function (**Tang and Gudas, 2011**).

The World Health Organization reports that vitamin A deficiency is the most prevalent cause of preventable childhood blindness. According to the WHO, ~250 M pre-school children suffer from vitamin A deficiency and ~10 to 20% go blind each year. Half die within 12 months of losing sight. Vitamin A deficiency also increases risk of illness and death from childhood diseases. A meta-analysis of the clinical literature of ~1.2 M children in 19 countries, predominantly in Asia, India, Africa and Latin America, concluded that vitamin A supplementation (children 6 months to 5-years-old) leads to a reduction in all-cause morbidity and mortality of non-hospitalized, disease-free children (**Imdad *et al.*, 2017**).

Generally, but not invariably, data from primary tumors reveal a correlation of Crabp1 and Fabp5 with poor prognoses (**Napoli, 2017**). For example, increased Crabp1 expression correlates with tumor virulence and reduced survival in multiple types of human breast cancers. A ratio Fabp5/Crabp2 mRNA expression ~4 in glioblastomas occurs in patients who survive ≤ 6 months compared to a ratio ~1 in patients who survive ≥36 months. Tumors of the short-term survivors expressed RAR target genes to a much lesser extent than the long-term survivors, whereas PPARδ/β-target genes were expressed robustly in short-term survivors.

Future Directions

As additional insight into the functions of retinoid binding-proteins and metabolic enzymes emerges, expanded clinical use of this information and retinoids themselves should follow. Possibly, selective drugs will be developed for specific clinical applications for each of the processes controlled by retinoids.

Closing Remarks

Vision depletes most vitamin A, but reproduction and post-natal life depend on the relatively small amount converted into RA. Even xeropthalmia, which causes blindness, results from a lack of RA, due to a lack of retinol. Carotenoids are plentiful in foods and retinol itself is inexpensive. Thus, vitamin A deficiency can be prevented. Its persistence results from societal issues.

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